Evaluation Of Single Contact Applications Of Iodophor Against The Fish Pathogen *Aeromonas salmonicida*, Cause Of Furunculosis

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Optimalization of survival among fertilized gametes is critical for the success of fish restoration programs that are limited by reduced population numbers. Iodophors are routinely used to disinfect fertilized fish eggs in order to ensure that surface pathogens do not kill the egg and to make sure that egg transfers between facilities do not transmit diseases. United States Fish and Wildlife Service (USFWS) Fish Health policy requires that eggs be disinfected in 50 mg/L of iodophor for 30 minutes at the facility where those eggs are fertilized. If the same eggs are shipped to a second facility, policy further states that those eggs must undergo a second disinfection in 100 mg/L of iodophor for 10 minutes at the receiving station. It was previously observed that when either treatment was applied individually, neither treatment could completely kill concentrations of A-layer positive *Aeromonas salmonicida* that exceeded $1.0x10^6$ colony forming units (cfu) of the pathogen. It was further noted that this prevalence occurred on Atlantic salmon (*Salmo salar*) eggs *in vivo*. At such prevalence, it was necessary to conduct both treatments in tandem in order to affect complete decontamination. Experiments conducted by the USFWS Northeast Fisheries Technology Center (Lamar, PA) also indicated that survival among Atlantic salmon eggs treated with concentrations of iodophor from 50 to 150 mg/L for up to 90 minutes was more adversely impacted by time of exposure. Relatively little mortality was attributed to the concentration of the iodophor, itself. This study was conducted to examine short-term, high concentration effects of iodophor against *A. salmonicida*. Times of exposure were conducted for 5, 10, 15 and 30 minutes with iodophor concentrations that ranged from 50 to 500 mg/L, respectively. This effort was designed to identify a single treatment regimen that was completely efficacious against high concentrations of *A. salmonicida* and could be further tested for safety among Atlantic salmon eggs *in vivo*.

A Polymerase Chain Reaction Assay To Monitor Channel Catfish (*Ictalurus punctatus)* Eggs For *Flavobacterium columnare*

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A newly developed species-specific polymerase chain reaction (PCR) assay was used to determine if *Flavobacterium columnare*, the causative agent of columnaris disease, was present in channel catfish (*Ictalurus punctatus*) eggs following spawning. This assay was compared to the standard culture method and was found to be as effective in detecting *F. columnare* in channel catfish eggs as the more traditional culture method. *Flavobacterium columnare* was successfully detected, in eggs that were artificially infected and in apparently healthy eggs, collected following spawning. The PCR assay was better than culture for detecting the bacterium in the naturally exposed samples. Further, PCR was used to show that povidone-iodine was effective in disinfecting egg masses of *F. columnare.*

Isolation And Characterization Of A Bacteriophage Which Infects *Yersinia ruckeri*

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Bacteriophages are viruses that infect and replicate in bacteria. While in theory these bacteria-specific infectious agents have great potential as potent prophylactic and therapeutic agents, little has been done to realize this potential. Here we present the isolation and partial characterization of a lytic bacteriophage (Phage YRNC10) with pathogenicity to *Y. ruckeri*, the causative agent of Enteric Redmouth disease (ERM). YRNC10 was isolated using an enrichment technique from sediments collected at a trout farm where outbreaks of ERM occur regularly. Nucleic acid analysis revealed that phage YRNC10 has a double-stranded DNA genome of approximately 60 kb. Spontaneous phage-resistant variants of *Y. ruckeri* have been identified which are sensitive to rainbow trout serum and are attenuated for virulence in rainbow trout. Several of these variants are defective in 0-antigen production and fail to bind phage, suggesting a role for the 0-polysaccharide in phage binding. Current investigations focused on assessing the effectiveness of phage YRNC10 for the treatment of *Y. ruckeri* infections and for the reduction of horizontal transmission of this pathogen.

Mycobacteriosis, Is It Truly A Chesapeake Bay Disease Or East Coast Problem

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The Maryland Department of Natural Resources, Fisheries Service, Fish and Wildlife Health Program continues to research and monitor mycobacteriosis in Maryland's portion of the Chesapeake Bay. Fish were collected in 2002 from the upper, middle, and lower areas of Maryland's portion of the bay. Additionally, fish were also collected from the Choptank River. One hundred and forty fish were collected and examined histologically for granuloma formation as an indication of mycobacteriosis. Fifty-one of these fish (36%) were infected. This percentage has remained in a range of 33-44% for five to six years. The disease is more prevalent in three to four year old fish. Personnel from the Virginia Institute of Marine Science (Gloucester Point, VA) and the National Fish Health Research Laboratory (NFHRL; Kearneysville, WV) have been doing similar work on the Chesapeake Bay, and there has been a great deal of good work accomplished; however, much more research and investigation remains to be done. Investigators at the NFHL started to adopt standard operating procedures in spring 2002; however, the results from the microbiology labs have been unclear. Therefore the resources from various agencies in this research need to be pooled in order to continue to carry out and improve the work toward a common goal. The NFHL has taken a lead position in getting all the parties working together in order to address the real issue, which is management of the striped bass stock. Stock assessment for the states is regulated by federal agencies. The National Marine Fisheries Service (NMFS) and Atlantic States Marine Fisheries Commission (ASMFC) regulate the states through fishery management plans (FMP). These FMPs incorporate a value for mortality, which is essential in regulating a stock. The fish health community needs to form an advisory group for the federal agencies to develop a figure for disease mortality of striped bass. This figure could then be incorporated into NMFS and ASMFC's FMP.

Detection Of Mycobacteria In Paraffin-Embedded, Formalin-Fixed Tissues Of Striped Bass (*Morone saxatilis*) With Peptide Nucleic Acid (PNA) Probes

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An epizootic of mycobacteriosis is currently occurring in striped bass (*Morone saxatilis*) of the Chesapeake Bay. Visceral and dermal granulomatous lesions characterize the disease, and infected fish are often significantly emaciated and/or disfigured. A large number of different mycobacterial species have been cultured from Chesapeake Bay striped bass, and it is likely that more than one contributes to production of disease. Reliable methods for detection and speciation of mycobacteria in fish tissues are therefore crucial to understanding the pathobiology of mycobacteriosis in the wild. Acid-fast staining is the standard method for detection of mycobacteria in histologic sections, but this method may lack sensitivity and does not allow for species-specific identification. Culture of mycobacteria does allow for limited species identification, but is very time consuming and labor-intensive, and is often logistically unfeasible in a field setting. As a first step toward developing rapid, convenient species-specific molecular detection techniques for mycobacteria in fish, we have designed and are validating genus-specific peptide nucleic acid (PNA) probes that detect mycobacteria in standard histologic sections. The PNAs are synthetic oligonucleotides in which standard bases (A, T, C, G) are attached to an uncharged glycine-derived backbone. The uncharged nature of the PNA molecule allows for improved penetration of hydrophobic membranes, such as the cell wall of mycobacteria. In addition, binding to target DNA is stronger and more specific than with standard oligonucleotide probes. Genus-specific probes were designed to target conserved regions of 16S *Mycobacterium* rRNA. These probes demonstrated strong, specific hybridization with *M. marinum* in tissue sections from experimentally infected striped bass and, in some cases, they appeared to have greater sensitivity than acid-fast staining. Optimization of these probes for other *Mycobacterium* spp. is underway.

Evaluating The Effects Of Oxytetracycline On The Microflora Of A Recirculating Aquaculture System

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According to the United Nation's Food and Agriculture Organization, aquaculture is the fastest growing food producing industry in the world. Because of this rapid development, and the shift towards more intensive farming methods, the aquaculture industry has encountered many viral and bacterial diseases, and the need for treatments to control and prevent disease. As a result, public health concerns have arisen about chemical residues and the development of antibiotic resistance in fish tissue. In the United States there are two antimicrobials approved by the Food and Drug Administration for aquaculture, oxytetracycline - Terramycin ®, and sulfamerazine - Romet-30 ®. These antibiotics are added to feed during the manufacturing process. Any food that is not eaten by fish, and the drug residues excreted in fish feces, will eventually reach pond sediments, or be captured in bio-filters used by recirculating aquaculture systems. Antibiotic residues are adsorbed in these sediments, which can create selective pressure on the microorganisms found in this environment. The purpose of this study is to survey bacteria found in recirculating aquaculture systems and to find a potential indicator organism. In addition to defining the bacterial isolation and identification methods, the isolated organisms will be tested for susceptibility to oxytetracycline following antibiotic susceptibility methods developed at CVM for aquatic organisms. Data from this study will help identify potential microorganisms to monitor for changes in antimicrobial susceptibility patterns following antibiotic use in recirculating aquaculture systems.

Mystery Of Epizootic Oral Tumors In Spring Chinook Salmon

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In December 2002, abnormal growths were observed among two-year-old Chinook salmon (*Oncorhynchus tshawytscha*) in a freshwater and a saltwater Pacific Northwest hatchery. Fish were collected in 2001 for a captive breeding program and they initially had red irregular formations along the jaw, tongue, and/or the roof of the mouth, which eventually progressed to large tumors that disfigured the lips and oral cavity. Microscopically, tumors were consistent with those of ameloblastic (tooth enamel-forming) origin: 1) multiple irregular clefts lined by palisading epithelial cells; 2) cords of epithelial cells contiguous with invaginations of the oral mucosa; and 3) areas of stroma that resembled stellate mesenchyme. By December 2003, tumor prevalence was 94% (n=131) and 100% (n=73) among salmon at the freshwater and saltwater hatcheries, respectively, but cohorts at these facilities were not affected. Tumors were also observed in the mouths of several Chinook salmon that had returned to natal streams during the summer of 2003. These fish were captured at weirs from three rivers in two watersheds, and at least one of these fish was of feral origin. Diagnostic procedures performed at Oregon Department of Fish and Wildlife to identify potential viral (and other) pathogens included: cultures from tumors and internal organs; transmission electron microscopy; polymerase chain reaction (PCR) assay for *Oncorhynchus* masou Virus and *Herpesvirus salmonis*; modified PCR for reverse transcriptase activity; injection of homogenates into Spring Chinook fingerlings; and cohabitation of fingerlings with tumor-laden adults. Investigations have not supported a viral etiology. A genetic cause was ruled out because relatedness between affected and unaffected fish was comparable to the relatedness among affected fish. Exposure to environmental carcinogens has not been dismissed; however, two site rivers are considered pristine environments, and a link among collection site contaminants was not established.

Coral Mortalities Along The Kenyan Coast Associated With Extensive Tissue Lysis: Possible Mycotic Etiology

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An apparent new source of coral mortality occurred along 600 km of coastline from Tanzania to Kenya in early 2002. *Astreopora*, *Echinopora*, and *Montipora* species were severely affected, with *Montipora* being nearly eliminated from Kenyan reefs. *Acropora*, *Platygyra*, *Goniopora*, and massive *Porites* were also affected; however, *Porites* and *Goniopora* rarely died and often recovered, whereas death for most other species occurred within two weeks. Scanning electron micrographs of affected corals revealed the presence of fungal filaments in affected samples. Examination of histopathology showed extensive necrosis and lyses of tissues in the three genera most severely affected by the syndrome. Special staining of tissue sections also revealed more fungal filaments in these genera than unaffected genera; however, the role of fungi in the Kenya coral mortalities is uncertain.

Catch And Release: The Importance Of TLC

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In May 2003, a moribund muskellunge with external skin lesions was observed in Lake Wissota, an inland lake in west central Wisconsin. The fish was netted, euthanized and frozen prior to necropsy. Fish length was 930 mm. The legal size limit for muskellunge is 850 mm in Lake Wissota. The fish had several well defined oval to circular lesions on the caudal peduncle and midbody of the fish. Skin was missing from some lesions, exposing inflamed and hemorrhagic muscle. Although the fish had been frozen, cultures were made from the lesions (at the margin of skin and muscle), kidney, spleen and liver on trypticase soy agar (TSA); the margin of the lesions, and pieces of kidney, liver, and spleen were fixed in 10% neutral buffered formalin for histology. Clear red ascites was present in the body cavity, but this could have been an artifact from freezing. There were no other significant signs in the viscera and no evidence of previous hooking injury. Bacterial cultures were submitted to the Wisconsin Veterinary Diagnostic Lab. *Streptococcus/Entercoccus* sp. were cultured from the kidney, liver and spleen. Sections for histopathology were stained with hematoxylin and eosin (H&E) and Giemsa. Giemsa stained sections showed tremendous concentrations of long, rod-shaped bacteria in areas of the muscle and the muscle appeared edematous. Melanomacrophage centers were plentiful in the kidney, and there were few intact tubules, which may have been an artifact of freezing or a consequence of bacterial infection. We would like some feedback on interpreting histopathology when tissues are first frozen. The locations of the lesions approximate the area where an angler would have held the fish while being photographed. Muskellunge fishing is considered trophy fishing in Wisconsin and most fish are caught and returned back to the water (after photos are taken, of course!). Anglers should take care when handling all fish to prevent serious trauma and subsequent bacterial infections.

First Record Of *Renibacterium salmoninarum* Isolation From The Sea Lamprey *(Petromyzon marinus)*

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Bacterial kidney disease, caused by *Renibacterium salmoninarum* is a serious widespread problem of salmonid fish species. It is currently unknown how high levels of this bacterium are maintained in the Laurentian Great Lakes' basin leading to infection prevalence of unprecedented magnitude. Research involving reservoirs and mechanisms of *R. salmoninarum* transmission in fish is lacking due to ecological complexity of heterogeneous habitats. Surprisingly, we isolated *R. salmoninarum* from the kidneys of the widespread fish parasite, the sea lamprey. The organism was cultured from 12% of Lake Ontario's lampreys examined and colonies were verified by *p*57 gene sequencing. It is highly probable that nonlethal lamprey encounters play a role in the temporal and spatial spread of *R. salmoninarum* in the Great Lakes basin.

A Case Of Severe Helminth Parasitism In Fish In A Small Impoundment

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A case was received that consisted of centrarchids from a 22 acre impoundment in the lower Hudson River Valley of New York State. The fish kill was acute in nature and included almost 100% of the fish in the impoundment. Water quality parameters associated with the fish kill did not reveal any problems. A wooded area surrounded the impoundment, with a few private residences set approximately 200 feet from the water's edge. There were no immediately evident sources from which pollutants or toxic substances could have entered the water. Fish submitted in October 2003 for examination consisted of bluegill *Lepomis macrochirus* (n = 3), redbreast sunfish *Lepomis auritus* (n = 1) and largemouth bass *Micropterus salmoides* (n = 2). A subsequent submission of 1 additional bluegill was made three days after the initial submission. Upon examination, the most significant findings were severe infestations of the internal organs by encysted digenetic trematodes (*Neascus*), encysted nematodes (*Eustrongylides*) and larval cestodes (*Proteocephalus*). Parasites made up approximately 50% of the combined organ mass of some fish. A most remarkable finding was that when the mesenteries were removed from the fish and examined with a stereomicroscope, the warming of the specimen by the illumination light appeared to activate the encysted nematodes, which perforated their cysts and began to migrate over the organ mass. Meteorological records indicated that a warm spell had occurred immediately prior to this event. Although we could not definitively attribute the mortalities to the parasites, we hypothesized that the rapid warming of the water in this shallow impoundment (mean depth = 5 feet) might have caused the nematodes and cestodes to become active and migrate within the fish, which caused their deaths.

An Unusual Case: Walleye Lesions In The Visceral Cavity

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An unusual disease condition was found in a 1.8 kg walleye from Merritt Reservoir in northwestern Nebraska. The fish was processed at the Stuttgart diagnostic laboratory in May of 1990, and when the body cavity was opened hard cysts or nodules in the swimbladder and visceral fat were observed. The nodules appeared to be formed around a worm-like mass. The nodules (10±5 mm in diameter) were composed of five to seven layers of tightly packed granules, that were at least partially calcified and the layers were held together by a sticky matrix. The outer coat of the nodule was convoluted with many protuberances. This is a first report of such cysts from fish and they appear to be similar to nodules induced by worms (encysted nematodes) in the intestines of swine and ruminants. Histopathological results were not conclusive but supported results found from the original diagnostic workup.

A Serendipitous Finding While Screening Spotted Musky for *Piscirickettsia* sp.

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In 2002, *Piscirickettsia* sp. was diagnosed in wild spotted muskies from Lake St. Clair. Prior to this diagnosis, fertile eggs from this population were transferred to the Wild Rose Hatchery in Wisconsin where they were hatched and reared for stocking as fall fingerling. A subsample, (about 200 fish), were held over winter with the intent to stock the fish in fall 2003. In July 2003, kidney and spleen from ten spotted muskies were aseptically removed and prepared for tissue culture. Severe cytopathic effects were observed in FHM and CHSE cell lines inoculated with samples from three fish, however *Piscirickettsia* sp. was not detected using the polymerase chain reaction (PCR) method. Electron microscopy revealed the presence of an icosahedral virus in two of the three samples and unusual viral particles in the third sample. Current investigations aim at characterizing the isolated viruses and determining potential sources of infection.

Abnormal Growth Associated With An Intracellular Bacterium In The Yellow Perch (*Perca flavescens*)

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A relatively large, lobulated tissue mass was noticed in the abdomen of a yellow perch caught from the Big Cedar Lake, Barry County, Michigan. This unusual mass occupied most of the abdominal cavity, pushing the visceral organs anteriorly and was loosely attached to the abdominal wall. In stained tissue sections, the mass consisted primarily of macrophage-like cells arranged in cords. Darkly stained rounded, 1-2 µm intracellular bodies were abundant. Transmission electron microscopy revealed that these intracellular bodies were rickettsia-like bacteria, which were enclosed within double membranes. The nature of this bacterium and its relationship to the unusual outgrowth are currently unknown.

Introduction To Largemouth Bass Virus

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Largemouth Bass Virus (LMBV) is an iridovirus that causes disease principally in largemouth bass. The disease caused by LMBV occurs during the summer and typically affects adult fish, which lose equilibrium and are found floating at the water surface. Largemouth Bass Virus was first isolated during 1991 in Florida and has now been found in several bodies of water in the eastern United States. Fish kills have occurred in some infected populations of largemouth bass, but in other locations this virus is found in largemouth bass and other fish species without overt disease. This virus appears to be the most likely cause of changes in largemouth bass populations in some reservoirs. Few problems with LMBV have been documented in hatchery-reared fish, but wild fish moved to hatcheries sometime experience rapid increases in prevalence of infection and viral concentration. This virus can replicate in a wide range of cultured cells and is easily isolated from infected fish. However, diagnosis of LMBV disease can be difficult because pathognomonic lesions are not present, concurrent bacterial infections are common, and isolation of LMBV is not sufficient to indicate that LMBV disease is the cause of morbidity.

Development Of Non-Lethal Testing Methods For Largemouth Bass Virus

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Although Large Mouth Bass Virus (LMBV) was first isolated from moribund largemouth bass (*Micropterus salmoides*) from Santee-Cooper Reservoir in South Carolina in 1995, archived samples showed that LMBV was isolated from bass in four Florida lakes as early as 1989. Since these reports, LMBV has caused many fish kills throughout the southeast and midwest. It has been suggested the virus has a predilection for larger "trophy" sized animals and that it can affect population size structure because trophy fish are impacted. Because of these impacts, it is important to establish strategies that prevent the spread of LMBV. Managers must have information on the prevalence and distribution of the virus, as well as it's overall impact on the population dynamics. The majority of health assessment tools require sacrifice of animals. Gathering such data will require collection of hundreds of samples of various sized animals from hundreds of locations that could have long-term effects on the population. Lethal sampling also makes it impossible to follow individuals or potentially, the population over time. While most individuals recognize it is necessary to sacrifice some animals for the greater good of a species, destructive sampling creates public relations problems if either large numbers of animals are sacrificed or if larger animals are killed. Such techniques also serve as a focal point for animal rights groups, which are becoming increasingly aware of fishery issues. By contrast, non-lethal techniques would allow for collection of statistically relevant samples and minimize the number of fish that would be lost. Finally, these techniques may potentially save time, cost less, and require less expertise, making them more cost effective than traditional techniques. This study evaluated the suitability of non-lethal sampling for the detection of LMBV. Non-lethal samples collected included blood, skin scrape, fin clip, and gill biopsy, which were compared to the standard kidney/spleen samples. All samples were assayed by viral isolation. In addition, total white blood cell counts were completed and the presence of anti-LMBV antibodies in serum was determined by agar gel immuno-diffusion.

Host, Pathogen And Environmental Factors That Affect The Susceptibility Of Largemouth Bass To Largemouth Bass Virus

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Populations of largemouth bass vary widely in their responses to infection with Largemouth Bass Virus (LMBV). Some populations experience epidemic mortality events (fish kills) while others remain apparently healthy. The reasons for this variation are poorly understood, but may hinge on factors operating (and interacting) at the level of the host, pathogen and environment. Preliminary epidemiological analyses indicated that fish kills tended to occur in warm seasons and within intensively managed water bodies. Mortality patterns were similar for water bodies in the same watershed. In a series of experiments, we explored these risk factors in greater detail using juvenile largemouth bass that were held in tanks and exposed experimentally to LMBV via intraperitoneal injection. Analysis of different isolates of LMBV indicated that multiple viral strains exist, differing both genetically and in their virulence. Outbreeding depression had a strong effect and was evident by the fact that hybrid bass suffered higher mortality from LMBV than non-outbred parental stocks. Ambient temperature was a significant risk factor: fish held at 30^{o}C died more quickly than fish held at 25^{o}C, and had higher viral loads in tissues. Water quality affected host survival in complex ways. Dissolved nitrate levels were paradoxically protective (higher nitrate levels in tanks were associated with reduced mortality and viral loads). Social stress from crowding was associated with increased mortality and viral loads. Stress from a single, maximally stressful experimental angling event, however, did not affect mortality rates or viral loads of infected bass. These results indicated that host responses to LMBV infection were modulated in complex ways by multiple, factors operating at the level of the host, pathogen, and environment. Understanding the effects of these factors will be critical in order to predict the impact of LMBV on wild populations.

Application And Implication Of Serology For Largemouth Bass Virus Infection Of Largemouth Bass (*Micropterus salmoides*)

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We investigated the pathogenicity of Largemouth Bass Virus (LMBV) in captive juvenile largemouth bass and the dynamics of the humoral immune response as measured by the agar gel immunnodiffusion (AGID) method. In experimental studies, 96 juvenile bass, held at either 23° or 29°C, were inoculated intramuscularly with $2x10^6$ infectious units of LMBV. Uninoculated "sentinel" bass were co-habited with them. Forty-eight control largemouth bass, inoculated with virus-free cell culture fluid, were also held in two separate tanks at the two temperatures. At 29°C, bass inoculated with LMBV developed severe necrotic lesions at the site of inoculation, whereas those held at 23°C developed comparatively mild lesions. Although none of the sentinel fish showed visual evidence of disease, lateral transmission of infection, as evidenced by AGID seroconversion, was much more efficient at 29°C (8/13; 62%) than at 23°C (1/11; 10%). Lateral transmission at 29°C occurred over a 3-month interval, suggesting that there was long-term shedding of virus. Precipitating antibody was detected in experimentally inoculated bass within three weeks of inoculation and was detected for over nine months in some bass held at 29°C. No controls developed pathologic or antibody responses. Re-challenge of seven recovered bass with the same dose of the original virus demonstrated that these previously exposed fish were resistant to the pathogenic effects of the virus, even at 29°C. None of these recovered fish developed lesions, whereas five similarly challenged naïve bass developed necrotic inoculation site lesions and two died. Persistence of precipitating antibody along with seroconversion of sentinel bass after several months of exposure to inoculated fish, especially at 29°C, suggested that some bass might not clear the viral infection, might become latently infected, and might shed virus under conditions of stress, such as high ambient water temperature. Our studies help validate that detection of precipitating antibody to LMBV is a simple non-lethal means to screen for evidence of exposure to the virus. Bass with precipitating antibody to LMBV may harbor the virus as a latent infection, subject to reactivation and viral shedding during times of environmental or other stress.

Expansion Of Largemouth Bass Virus Range In Michigan's Lower Peninsula Lakes

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Largemouth Bass Virus (LMBV) is a ranavirus (Family *Iridoviridae*), originally isolated from largemouth bass (*Micropterus salmoides*) in the Santee-Cooper Reservoir, SC, in the mid-1990's. In less than a decade, LMBV spread into at least 17 states in the southeastern and midwestern United States. In 2000, LMBV infection was detected from Lake George, a border water between Michigan and Indiana. By 2002, LMBV had been confirmed in 23 Michigan lakes with suspected LMBV related mortalities in 10-15 other lakes. In the summers of 2002 and 2003, the Michigan Department of Natural Resources (MDNR) initiated an LMBV sampling program. Adult largemouth bass were collected from 32 inland lakes by MDNR and the Indiana Department of Natural Resources collected adult largemouth bass again from Lake George for LMBV testing. These lakes represented the western, eastern, and central zones of Michigan's Lower Peninsula radiating out from the initial confirmed site. Results indicated that there was a progressive northward, westward and eastward expansion of LMBV's range in the southern region of Michigan's Lower Peninsula since it was initially reported in Lake George. Sequence analyses of the major capsid protein and methyltransferase genes of the five isolates demonstrated their homology to each other and to the LMBV strain isolated from the Santee-Cooper Reservoir. While the impact of this virus on largemouth bass populations has been limited in most cases, there is a large amount of uncertainty about the pathogen and there is cause for concern for the health of North America's most popular sportfish species.

Cryptobiosis In Cichlidae: Fun For The Whole Family

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Granulomatous gastritis is common in numerous species of Old and New World ornamental cichlids. Nutritional disease, clostridiosis, mycobacteriosis, and flagellated protozoal infections are a few proposed or identified etiologies. Fungi, rickettsia, amoeba, nematodes, and foreign bodies can also incite similar responses. Specific etiologies have been difficult to prove. Based on diagnostics and observations from clinical cases, and on the scientific literature, infection with *Cryptobia iubilans* appears a major cause of granulomatous gastritis/gastroenteritis in cichlids. *C. iubilans* and *Spironucleus vortens,* both flagellated protozoans found in the gastrointestinal tract in cichlids, differ in morphology, typical pathology, primary target organ, and therapy. *S. vortens* does not cause granulomatous disease, targets primarily the intestinal tract, and can be treated effectively in ornamental tropical fish with metronidazole. *C. iubilans* causes primarily granulomatous gastritis; severe infestation can result in more widespread, systemic granulomatous disease, and metronidazole is not an effective treatment. Morbidity and mortality in a population seem affected by many factors: water quality, presence of other pathogens, diet, fish species, size, and age. Good husbandry appears important for prevention and severity reduction. Rigorous diagnostics (including TEM) are important for detection and identification of the parasite, which has been found alive with in cell vacuoles and dead within granulomas. Overall epidemiology is poorly understood; in more advanced disease, mobile, luminal parasites may no longer be present. Bath treatments of dimetridazole (at 80 mg/L for 24 hours x 3 days) and 2-amino-5-nitrothiazol (at 10 mg/L for a minimum of 3 days), in experimental studies, appear to be promising treatments against this parasite, reducing prevalence of infection, although further studies are necessary.

Fusariomycosis In Captive Parrotfish (Family *Scaridae*) And Bonnethead Sharks (*Sphyrna tiburo*)

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Fusariomycosis, caused primarily by *Fusarium solani*, has been identified as suppression of immune function in these species and/or the provision of favorable growing temperatures for the fungus. Parrotfish developed fusariomycosis because they were maintained at lower water temperatures than those in their native range. The bonnethead sharks developed disease following multiple acute low temperature events whereby in-line heater failure resulted in water temperatures dropping below 24 °C (19.9-22 °C) for 1-2 days at a time. To date, treatment of parrotfish and bonnethead sharks has been unsuccessful due, in part, to lack of early recognition of the primary pathogen and failure to attain therapeutic anti-fungal drug levels in plasma. Recent successful treatment of a balloonfish (*Diodon holocanthus*) with aggressive surgical debridement procedures and an extended course of fluconazole provided encouragement for the future treatment of individual cases of fusariomycosis. a significant cause of disease in captive marine fish. At Disney's Typhoon Lagoon and Living Seas Pavilion, bonnethead sharks (*Sphyrna tiburo*) and parrotfish (Family Scaridae) have historically been highly susceptible to infection. Fusariomycosis has accounted for at least 30% of all parrotfish mortalities. Initially, skin ulcerations and loss of scales and/or epidermal tissue on the body and/or jaws were noted. Progression into skeletal muscle and, subsequently, the viscera occurred in most cases. Skeletal destruction also occurred in some cases. Histological evaluation (H & E stain) demonstrated fungal hyphae in most affected tissues. In bonnethead sharks, fusariomycosis developed in 4/6 animals that entered quarantine. Mortality occurred over approximately four months. All animals presented with cutaneous pustules and/or ulcerations associated with the cephalic and the lateral line canal system. Although histopathology revealed lesions in multiple internal organs, fungal invasion and associated damage was limited to the skin, subcutaneous space, and adjacent skeletal muscle. Involvement of the gills was also observed in one case. While trauma and dietary deficiencies may have contributed to development of fusariomycosis, thermal stress due to low temperatures was felt to have precipitated development of the disease.

Surgical Cases In Ornamental Fish: A Chance To Heal With Cold Hard Steel

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Ornamental fish can be excellent surgical patients. A large percentage of these animals are nishikigoi, or simply koi (*Cyprinus carpio*), which are taxonomically an ornamental carp. Most small and exotic animal hospitals will already possess the necessary surgical instruments and other supplies necessary to perform surgery on fish. A delicate or ophthalmologic pack is helpful when working with small patients. Some of the reasons fish may require surgery include: Traumatic injury from a predator or fixed object in a pond or aquarium; dystocia (egg-binding); neoplasia (either external or internal); enucleation of a diseased eye; foreign body removal; swimbladder repair or removal; surgical biopsy of an internal or external tissue; and repair of a torn fin. Listed are some general considerations when performing surgery on a fish that may differ from surgery on a terrestrial animal: 1) The skin should be kept moist throughout the surgical procedure. During prolonged procedures, a red rubber catheter and large syringe can be used to carefully moisten the fish patient without splashing water into the incision site. 2) Patient monitoring can be performed with a pulse Doppler or properly placed ECG leads. 3) A clear plastic avian-style surgical drape is advantageous. The plastic helps retain moisture around the fish, does not allow moisture to leak through and compromise the surgical field, and provides a sterile working surface. A rim of petroleum jelly can be used to adhere the drape to the fish if desired. 4) Surgical preparation should minimize disruption of the skin and mucus, because these are major barriers to infection. A simple swipe along the intended incision site with a cotton swab soaked in sterile saline, or at most dilute povidone iodine or chlorhexidine solution, to reduce gross contamination suffices in place of a traditional surgical scrub. 5) Removing scales along the incision line facilitates a smooth entry. 6) Retractors are a valuable tool for visualizing and accessing various internal organs. 7) Bipolar cautery works well for hemostasis. 8) Needles with a cutting tip facilitate skin penetration (skin is the holding layer in fish). 9) A variety of suture types are used with fish but synthetic monofilament types work best.

Piscirickettsiosis-Like Syndrome In Tilapia

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An epizootic disease affecting tilapia in both marine and fresh water ponds was reported in 1992 in Taiwan, Hawaii in 1994, sporadically throughout the world during the 1990's and the United States in the last few years. All species of tilapia were affected by the disease. Mortality was greater than 75% in severe outbreaks and averaged around 30%. Diseased tilapia often swam erratically and had trouble staying at depth. Upon examination, the fish exhibited lethargy, occasional exophthalmia and petechial skin lesions. The gills were often extremely necrotic with a patchy white and red appearance. Grossly the spleen and kidneys were extremely granular in appearance with whitish irregular nodules throughout. Microscopically, granulomatous infiltrates were observed throughout the viscera with the exception of the liver. The granulomas contained organisms that were Geimsa positive, acid-fast negative and weakly Gram negative. Transmission of the syndrome was produced when naïve tilapia were co-habitated with diseased tilapia. The syndrome has been associated with cold stress and poor water conditions.

Whole Blood and Plasma Cholinesterase Levels In Normal Koi (*Cyprinus carpio*)

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Organophosphate and carbamate insecticides produce toxic effects by binding with cholinesterase enzymes, causing inhibition of acetylcholine catabolism. Excessive acetylcholine levels cause excessive synaptic neurotransmitter activity. In most animal species, whole blood is the preferred sample for evaluation of cholinesterase activity. Previous reports of cholinesterase activity in carp have involved sampling of serum, plasma, brain, liver, muscle, and heart. Whole blood samples were obtained from 12 healthy koi (*Cyprinus carpio*). Whole blood cholinesterase activity was measured in all 12 samples. Because one sample was clotted, it was centrifuged and the analysis was performed primarily on plasma. To evaluate if this may have significantly affected the result, plasma cholinesterase activity was also measured in four additional samples. The average of the whole blood cholinesterases was 0.08 mcmol/ml/min (SD=0.024). With the value from the clotted sample removed, the average became 0.075 mcmol/ml/min (SD=0.019). The results for the plasma cholinesterases were not very different, with an average of 0.08 mcmol/ml/min (SD=0.012). Since these reference values are close to the minimum detection limit for the method of analysis used, evaluation of blood samples from koi suspected to be suffering from anticholinesterase toxicity may be difficult.

Ichthyophthiriasis: Atypical Outbreak InTwo Susceptible Ornamental Fish Species In Egypt Under The Same Environmental Conditions

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Ichthyophthiriasis is one of the most devastating diseases that affects freshwater ornamental fish. An outbreak of Ichthyophthiriasis erupted in one of the aquaria holding Siamese Shark (*Pangasius sutchi*) and Goldfish (*Carrasius auratus* var. *bicausatus*) at the College of Veterinary Medicine, Cairo University, Egypt. Initial observation of the outbreaks showed that only *Pangasius sutchi* was affected with typical white spots associated with mortalities. However, *Carrasius auratus*, a species susceptible to Ichthyophthiriasis, in the same aquarium showed only mild erythema that disappeared during the course of infection with no mortalities. To confirm the previous observation an experimental infection with Ichthyophthirias was induced in *Pangasius sutchi* and a similar number of goldfish was introduced to the aquarium one day later. Three days after the induction, *Pangasius sutchi* started showing typical clinical signs. Mortalities associated with severe infection were recorded in *Pangasius sutchi* by the seventh day after infection. *Carrasius auratus* showed only mild erythema that disappeared by the end of experiment. Histopathological examination of skin from both species in natural and experimental infections was performed and will be addressed. Substantial numbers of typical large size trophonts surrounded by layers of fibrous tissue, melanophores and hemorrhages were detected in dermal and epidermal layers. Underlying myodegeneration was also associated the skin lesions in *Pangasius suchi*, but *C. auratus* skin showed only few numbers of small trophont with prominent hemorrhage in dermal and epidermal layers. No fibrous layer formation or myodegeneration was detected in *C. auratus* skin.

From Wild To Exhibit: Notes On Caribbean Collecting, Shipment And Quarantine

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Because of various constraints and limited knowledge on reproduction for marine aquatic animals, public aquaria often conduct collecting trips to stock exhibits with live specimens for display purposes. Although these displays are designed for public education, collecting animals poses a mixed bag of issues that must be addressed. Many issues are logistical, environmental, and/or political. At the New England Aquarium, we conduct one or two Caribbean collecting trips annually in order to diversify species in our largest premier exhibit tank, the Giant Ocean Tank (GOT). This tank resembles a Caribbean coral reef, showing animals that inhabit Caribbean waters. The GOT exhibits one of the most diverse Atlantic collections in the world with over 120 species and 600 + specimens of fish and 3 species of sea turtles. These inhabitants vary in size (cm to m) and in age ranges (span 1- 25 + years). Some animals in this tank such as the Pacific batfish, *Platax orbicularis*, are marquee for educational programming, as they illustrate the dangers of releasing non-native species into Atlantic tropical waters. The prominence of the New England Aquarium in conservation, research and education, have lead staff in the husbandry department to constantly improve collecting techniques for obtaining specimens. This presentation follows Caribbean fishes through wild collection, during transport, in quarantine, and onto exhibit to illustrate various protocols, techniques, apparatus, programs, and methodologies that are required to successfully maintain and stock exhibits in public aquaria. Collaboration between our Fishes and Animal Health Departments, as well as with other institutions, serves to continually adapt, evolve, and improve this program, which has achieved minimal mortalities (1-3%) and provided a remarkable educational centerpiece for the New England Aquarium over the last 35 years.

Diagnostic Microbiology For Small Laboratories: A Practical Guide For The Identification Of Bacterial Isolates From Fish

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Diagnostic microbiology is an important health assessment tool for veterinarians in aquatic medicine. However many aquaculture facilities, zoo, and aquarium laboratories are limited in staff and microbiology expertise. Time and budget constraints may prohibit submission of cultures to a reference lab. Biochemical tests have been the standard for bacterial identification for years, but results may yield incorrect identifications, especially for oxidase-positive, non-lactose fermentors. Analyzers developed to automate biochemical techniques for identifying bacteria from humans are of limited use with isolates from fish and method validation is lacking. There is a need for the combination of biochemical tests with more advanced techniques to offer accurate, efficient results. In 2001, the authors reviewed the identification data from over 700 bacterial isolates found in clinical cases at the National Aquarium in Baltimore (NAIB) and developed a protocol, organized in a series of easy-to-use flowcharts, to group the bacteria into clinically useful categories. The bacteria were grouped as follows: Gram-Positive Coccus (*Staphylococcus* sp., *Micrococcus* sp., *Enterococcus* sp., *Streptococcus, Non-Enterococcus*); Gram-Positive Bacillus (*Listeria* sp., *Corynebacterium* sp., *Erysipelothrix, Bacillus* sp., *Clostridium* sp., *Carnobacterium* sp., *Actinomyces, Mycobacterium/Nocardia/Tsukamurella*); Gram-Negative Cocco-Bacillus (*Moraxella* sp., *Acinetobacter* sp.); and Catalase- and Oxidase-Positive Gram-Negative Bacillus (*Vibrio/Plesiomonas/Sphingobacter, Aeromonas* sp., *Shewanella putrefaciens, Pseudomonas aeruginosa, Pseudomonas (*non-*aeruginosa)/Flavobacter*). Oxidase-negative, Gram-negative bacteria were identified by biochemical tests and commercially available test strips. Isolates were frozen at –70 °C and, when further identification was required, subcultured for submission to a reference laboratory specializing in aquatic microbiology. This strategy has been in place at NAIB since 2001; it offers an efficient and inexpensive option for preliminary identification of isolates that is easily taught to laboratory staff with basic microbiological skills.

Molecular Approaches To The Diagnosis Of Infectious Disease Concerns In Commercial Aquaria

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Infectious diseases are among the most significant and costly threats to the health of individual fish species and tank populations in commercial aquaria. As attempts are made to maintain collections of greater numbers and more diverse species of aquatic animals, the incidence of infectious disease in aquarium populations is bound to increase. A potential complicating factor in this equation is the concern that newly encountered or emerging diseases in aquarium-held fish may be caused by microbial agents that are difficult, if not impossible, to isolate by routine microbiological techniques. Specimens for microbiological analysis may not be easily obtained in some instances because of limitations imposed by species rarity, specimen size, or husbandry conditions. Molecular biological techniques have provided valuable insights into identification and characterization of unculturable infectious agents in human medicine. Such approaches can be useful in identification and characterization of fastidious or yet uncultured infectious agents in host species economically relevant to commercial aquaria. Members of the Syngnathidae, e.g. seahorses, pipefish, and seadragons, have been subjects of intensive aquaculture initiatives in aquaria nationwide. These are prominent exhibit species in which several infectious diseases have emerged and threaten their long-term maintenance in aquaria. A concerted approach coordinating histopathologic analysis of tissues with consensus sequence-based PCR, targeting conserved genes, has provided partial gene sequence data from which genus- or species-level identifications of infectious agents can be made in the context of anatomic correlates of disease. Conscientious and controlled application of molecular approaches using consensus sequence-based PCR may advance identification and characterization of certain fastidious or yet uncultured infectious agents and contribute to their epidemic control.

Apatite Concretions In Spiral Valve Of Yellow Stingray (*Urobatis [Urolophus] Jamaicensis*) Fetal Pups

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Yellow stingrays conceive in captivity, but rarely proceed to normal parturition. Retained fetuses can mummify and cause life-threatening complications. During clinical management of five yellow stingray females housed intermittently with one male, radiography and ultrasonography were used to stage fetal development. One striking radiographic feature of the fetal pups was a beehive-shaped radio-opaque spiral corresponding to the spiral valve. By contrast, the spiral valve was not radiographically detectable in the adults. The spiral valve was the only radiographic feature visible in early-term fetuses. In larger fetuses, the barb, vertebral column and pectoral girdle became more radiographically apparent, as the mineralized opacity of the spiral valve diminished asymmetrically. The fetal spiral valve was a useful ultrasonographic landmark that was identified by its oval shape and alternating thin wavy hyperechoic and hypoechoic bands. Assisted parturitions were performed twice by manual dilatation and extraction, resulting in two premature pups and a normal-appearing full-term pup that died 24 h later. Pharmacologic induction was performed using intracloacal misoprostol, resulting in delivery one hour later of five pups that thrived at 2 months. Confirmed or presumed spontaneous abortions occurred three times and caused concern about the stress of handling, transport and anesthesia. In formalin-fixed specimens, the spiral valve was gritty and difficult to cut, particularly in smaller fetuses. On cut surface, grey concretions with varying amounts of soft pale green gelatinous material were interleavened within folds of the valve. After routine processing for histology, the organ was easily cut with the microtome. Histologically, grey granular material and homogeneous pale pink glycoprotein-like material filled the spaces between spiral valve folds. Cytoplasm of the epithelial cells was uniformly distended with pink material. Stone analysis of the concretions revealed that they were composed of 100% apatite ($Ca_5[PO_4]_3[OH]$ and related compounds).

Problems With Protozoa: Treating A Large Reef Tank For *Cryptocaryon irritans*

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Since the New England Aquarium opened its Giant Ocean Tank in 1970, there have been unavoidable outbreaks of various pathogens including *Cryptocaryon irritans, Neobenedenia,* and *Amyloodinium* despite vigorous quarantine efforts. Some previous and historical treatments have included copper sulfate (various forms), trichlorfon, and praziquantel. Preventive measures have included the addition of cleaner wrasse fish; careful disinfection of wetsuits, nets, food prep materials, and other dive gear; stricter quarantine procedures; monthly surveillance of sentinel fish using freshwater dips, skin scrapes and gill clips; sediment filtering; and skimming with an egg catching device. In recent years, *C. irritans* has caused the most frequent outbreaks of disease in the Giant Ocean Tank. Such outbreaks have required three tank treatments in the last two years. Possible sources of infection include newly introduced fish, cross contamination with human or equipment vectors from other galleries or quarantine areas, the water supply from Boston Harbor, the presence of a copper-resistant strain of *C. irritans,* or aging infrastructure and deferred maintenance that has prevented theraputants to penetrate all the filters and gravel beds. The most recent treatment with Cupramine© (Seachem Laboratories Inc.), a copper product complexed with an amine molecule, although safe, was least efficacious. Although no mortalities were observed when Cupramine© was applied, subsequent reinfection after six weeks suggested that stages of the parasite persisted well beyond the termination of the treatment. This discussion hopes to demonstrate the trials, tribulations, and environmental quality management required to treat a 200,000-gallon system for a disease outbreak.

Characterization Of *Photobacterium damselae* Subspecies *damselae* Isolates From Teleosts And Elasmobranchs Housed In An Aquarium Exhibit

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Photobacterium damselae subsp. *damselae* (Pdd) is a marine vibrio that causes extraintestinal infections and gastroenteritis in humans and fatal septicemia and wound infections in cartilaginous and bony fish. Nine isolates, obtained from various fish species and sources, were identified using the API20E and MIDI identification systems and by a duplex PCR assay targeting concomitantly, a species-specific 16s rRNA gene sequence and the phospholipase D hemolysin (damselysin) gene. All of the isolates were positive for the 16s rRNA gene amplicon; none of the isolates were positive for the damselysin gene amplicon; yet 7 of the 9 isolates were hemolytic on sheep blood agar. All were urease positive indicating that these isolates were Pdd not *P. damselae* subsp. *piscicida*. Plasmid profile analysis showed that several of the isolates possessed plasmids; some had more than one. Pulsed field gel electrophoresis (PFGE) showed that the isolates comprised 8 different subtypes with Dice relatedness coefficients ranging from 40% to 100%. However, 2 of 9 isolates had indistinguishable PFGE banding patterns. These two isolates were obtained from ill fish housed in the facility, 15 months apart. Together, these data suggested that isolates causing infections in the aquaria exhibits arose with one exception, from different bacterial clonal linages; further suggesting that derivatives of no one clone dominated any ecological niche or aquarium location. Lastly, a non-damselysin hemolysin was isolated from one Pdd strain. The hemolysin was heat labile, had a molecular weight of 64,000 and an isoelectric point of ca 4.7. In summary, these studies also showed that the strains causing infections in the fish represented a diverse group, several isolates possessed plasmids, and that a second hemolysin that is different than the already described damselysin was isolated. It is not known whether plasmid(s) play a role in disease. Other studies will further characterize the hemolysin's role in disease.

Further Investigation Into The Brain Parasite In Yellow Perch

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Myxozoan parasites found in brains of yellow perch (*Perca flavacens*) presented to the Pennsylvania Animal Diagnostic Laboratory System in 2000 were compared to a case in September 2003 that affected juvenile yellow perch. In 2000, the affected fish were raised in recirculating systems and came from states where they were grown in earthern ponds. The fish showed hyperexcitability when introduced into the recirculating systems that changed to lethargic abnormal swimming and drifting with the water flow. Mortalities occurred 3-5 months post introduction to the recirculating systems. Gross and postmortem examinations performed were not remarkable except once when *Flavobacterium columnare* was isolated, but resolved with antibiotics. Viral isolation was negative. Histopathological examination revealed large localized clusters of spores beneath the ependymal lining of the central canal of the brain with little or no inflammatory response. Based on spore location and morphology, the parasite was identified as *Myxobolus neurophila.* In the 2003 case, fish did not show any clinical signs or mortality. Histological comparisons were performed using fresh and formalin fixed tissue and the parasite was only found in yellow perch and not in four other species (large mouth bass, fat head minnows, golden shiners, and walleye) that were raised in the same pond or shared the water supply. Spore visualization was enhanced with Diff-quick stain on touch impressions and Giemsa stain for histological preparations. Spores in unfixed tissues measured 15.4-16.8µm long and 5.6-7.0µm wide with two prominent polar capsules. Presence of an iodopholius vacuole with Lugol's iodine was not confirmed but spores picked up the stain, which facilitated differentiation from tissue in the touch impressions of the brain. As in 2000, spores of this histozoic myxosporea were found in the multiple locations in the brain including the meninges. A mild mononuclear inflammation was often present when spores occurred outside parasitic cysts. Spores (often immature) were also found in the spinal cord. The histopathological examination was performed on cross-sections of the fish and spores were not readily evident in other organs. Currently, other techniques (EM, PCR) are being employed to provide further information on this parasite.

The Discovery Of Four Cryptic Species Of Oligochaete Hosts (*Tubifex tubifex*) Provides Insights Into The Spread Of *Myxobolus cerebralis* Among Wild Trout Populations

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Two studies in Colorado examined river drainages where whirling disease, caused by *Myxobolus cerebralis*, has or has not had significant impacts on wild populations of trout. The distribution of oligochaete hosts resistant or susceptible to infections with *M. cerebralis* was determined at each site with newly developed mitochondrial 16S rDNA (mt 16S rDNA) lineage specific markers. We tested the hypothesis that certain cryptic species (mt 16S lineages) of *Tubifex tubifex* preferentially support *M. cerebralis* infections in a given geographic region. In the first study, worms from seven sites were experimentally exposed to the parasite in the laboratory prior to genetic screening. In the second study, worms collected from thirty sites were not exposed to *M. cerebralis* in the laboratory and these samples were screened shortly after field collection. Results from both studies demonstrated that genetically heterogeneous populations of *T. tubifex* or cryptic species coexisted at most sites, however non-susceptible *Tubifex sp.* (lineage V and VI) predominate at low impact sites. This supported the conclusion that when these non-susceptible *Tubifex* worms were in greater abundance the potential for more severe effects of whirling disease on wild trout populations may be ameliorated. Furthermore, the distribution and abundance of particular *T. tubifex* may in part be responsible for defining high compared to low impact whirling disease sites.

Bonamia Sp. (Haplosporidia) Found In Non-Native Oysters, *Crassostrea ariakensis,* In Bogue Sound, North Carolina

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The Suminoe oyster, *Crassostrea ariakensis*, is being considered for introduction into the middle Atlantic coast region of the United States, where diseases have decimated native *Crassostrea virginica* stocks. Triploid *C. ariakensis* produced at the Virginia Institute of Marine Science (VIMS) and transferred to Bogue Sound, North Carolina experienced high mortality in the summer of 2003. Histopathological examination of oysters collected after the peak mortality revealed the presence of intrahemocytic inclusions suggesting a *Bonamia* species-like parasite infecting hemocytes in 9.1% (2/22) of the oysters. Sampling of a subsequent, October 2003 deployment to Bogue Sound revealed 47% of the oysters were infected by a *Bonamia*-like parasite. Diagnosis of a second sample by *Bonamia*-specific PCR primers revealed a 60% prevalence and confirmed that the parasite was *Bonamia* sp. Sequence analysis of the PCR fragment suggested that the parasite was not one of the described species of *Bonamia*. No infections were detected in *C. ariakensis* from Pamlico Sound, North Carolina or from the York River, Virginia that originated from the same hatchery cohort, suggesting that the parasite originated in Bogue Sound and not from the VIMS hatchery. Additionally, no *Bonamia* sp. infections were detected in *C. virginica* from Bogue Sound, indicating specific susceptibility of *C. ariakensis* for the parasite. The natural host for this *Bonamia* sp. is unknown. How, or if, this parasite will affect the suitability of *C. ariakensis* for resource restoration or aquaculture in the middle Atlantic region must await studies on temperature and salinity tolerance, transmission and distribution of the natural host. Nonetheless, *C. ariakensis* appears to be highly susceptible to a previously unknown local pathogen.

Protozoan Infections Of The Eastern Oyster (*Crassostrea virginica*) In The Upper Chesapeake Bay: A Potential Ecological Forecast

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Perkinsus marinus and *Haplosporidium nelsoni* cause devastate populations of the eastern oyster, *Crassostrea virginica*, along the United States Atlantic coast and Gulf of Mexico. Salinity and temperature are major controlling factors in the prevalence and infection intensity of these two parasites. Fishery managers and oyster growers use this relationship to predict potential outbreaks of disease in oyster populations and to determine the best time, or sites, for planting and harvesting of oysters. Predicting medium or long term fluctuations in oyster disease in the Chesapeake Bay is limited by the inability to accurately predict medium and long term fluctuations in weather patterns such as occurrence and duration of drought conditions. Several mathematical models have been developed to assess the health of oyster populations in the Gulf of Mexico and Chesapeake and Delaware bays. An impediment to the accuracy of mathematical models is the current gap in knowledge regarding the parasites, the host, and their interactions. A prototype population dynamics model was recently developed by Jordan and Coakley to predict outcomes of management options for resource restoration and fisheries enhancement. Sixteen years of Chesapeake Bay oyster disease and population data collected by the Maryland Department of Natural Resources provided the basis of the model. The potential for using the Jordan and Coakley model to predict the effects of disease on oyster populations in the upper Chesapeake Bay was examined in this study. Alternatives to the stock assessment or population dynamics modeling approach to predicting oyster disease are also discussed.

The Depression Of Oxygen Uptake In Blue Crabs In Response To Bacterial Challenge

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We investigated the respiratory responses of the blue crab, *Callinectes sapidus*, to an injection of bacterial pathogen *Vibrio campbellii*. Current results suggested that bacteria were removed from the hemolymph in a process associated with a reduction in circulating hemocytes. It was thought that the gill was an important site of accumulation and ultimate removal of the bacteria. We tested the hypothesis that the accumulation of bacteria at the gills disrupted gas exchange. Whole animal oxygen uptake was measured in well-oxygenated water (25 ppt, 25°C) in control animals injected with saline and compared with animals injected with 2.5×10^4 live *Vibrio*/g crab weight. Injections were made directly into the lumen of the heart to promote rapid distribution of *Vibrio*. Crabs injected with *Vibrio* had a significantly ($p<0.037$, t-test) depressed oxygen uptake (2.56 µmol/g-h, 0.23 sem, n=6) compared with controls (4.53 µmol/g-h, 0.59 sem, n=6) in a period of 1 to 2 hours after injection. In separate experiments we compared pre- and postbranchial hemolymph Po_2 and pH in sham-injected controls with the same variables in *Vibrio*-treated crabs 30 min after treatment. Postbranchial Po_2 was significantly ($p=0.006$, t-test) depressed in *Vibrio*-treated crabs (99 torr, 5.8 sem, n=9) compared with the controls (122 torr, 3.4 sem, n=7). There were no significant differences in any of the other variables. Hemolymph pressure was measured in the infrabranchial sinus and the pericardial space unilaterally before, during and after sham and *Vibrio* injection, allowing for calculation of hemolymph pressure drop across the gill circuit. Ventilatory function, monitored as pulsatile hydrostatic pressure in the branchial chamber, did not change significantly after either sham or bacteria injection. Resistance to hemolymph flow increased across the gill circuit after bacterial injection. These results suggested that injection of crabs with *Vibrio* causes a disruption of gas exchange at the gills, supporting our hypothesis. The precise mechanisms involved were not yet clear and are currently under investigation. (Supported by NSF IBN-0212921.)

Effects Of Environmental Stressors On Mortality Rates In Lobsters: A Controlled Laboratory Study

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The cause of unprecedented lobster mortalities in western Long Island Sound (LIS) in 1998 and 1999 is under investigation by scientists who are conducting numerous studies on paramoebiasis, pesticide poisoning, and environmental stressors. The objective of our contribution to this effort is to determine whether increased (but environmentally realistic) conditions of temperature, hypoxia, sulfide and ammonia, alone or in combination, can increase susceptibility to microbial infections, and thus, affect mortality rates. A study completed in 2003 indicated that lobsters exposed to levels of hypoxia and hydrogen sulfide that are known to occur in LIS became more susceptible to the bacterial lobster pathogen *Aerococcus viridans.* Further experiments to evaluate high-temperature stress in the absence of bacterial infection showed that lobsters obtained from eastern LIS survived at 24^{o}C indefinitely under normoxia. Survival dropped to three days at a lower oxygen level of 95 µM, and to only one day when dissolved oxygen levels were further dropped to 55 µM, and combined with 9 µM sulfide and 17 µM ammonium.

Immunodiagnostics In Theory And Practice

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Current capabilities in immunodiagnosis transcend those most routinely exploited by fish health professionals. Although some diagnoses can be sensitively ascertained by molecular techniques, a number of prognostic indicators of immunity or immunopathology are determined via immunoassays. However, as with all diagnostic assays, optimal utilization of immunoassays requires in-depth knowledge of the system being examined. Often antibodies are developed with the purpose of being diagnostic of a disease or physiological condition without knowledge of the specific antigen or the specificity of the antibody or how either relates to the disease. For example, the relevance of the level of antibody activity generated or even the presence of the antibody itself has been confused with a prophylactic state, without consideration of the fact that antibodies may, at times, be contraindicative of a prophylactic response. Currently immunoassays have progressed beyond the simple assessment of antibody activity to the determination of the form of antibody induced, its specificity, and whether a cellular response has been generated. Thus the role immunodiagnostics has not only expanded in its sensitivity and specificity, but now also includes more circumspect analyses of other parameters of the immune response.

Molecular Diagnostics In Theory And Practice

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Developments in molecular genetic technology and the proliferation of sequence information during the past 20 years have facilitated progress in several areas of aquatic disease research. Advances have been made in pathogen detection and identification, as well as the use of molecular tools to enhance studies of transmission, host/parasite interactions and mechanisms of virulence. There is an expanding database of molecular information available, which can be used to develop sensitive and specific assays. Molecular diagnostics, including DNA probes for in situ hybridizations and primers for polymerase chain reaction (PCR) are available for a variety of pathogens found in the aquatic environment. These methods greatly facilitate disease diagnoses, especially when it is difficult and/or time-consuming to isolate and identify pathogens, and in situations when pathogens are not easily distinguished morphologically. In addition, molecular diagnostics are invaluable when a particular species within a genus or even certain strains of the same species may be pathogenic, while closely related species or strains are harmless. Although molecular techniques are powerful, caution must be employed to ensure that assays are based on adequate molecular information and that the information and techniques are used appropriately. It is essential that molecular probes and primers are reliable, accurate and sensitive. Development of genus-specific, species-specific and/or strain-specific DNA probes and PCR primers entails obtaining sequence data for an intended target locus from as many different strains and species within a genus as possible. In addition, DNA sequences from targeted loci are needed from closely related taxa. Intra- as well as inter-specific sequence variation must be characterized. This approach optimizes the chance of developing a genus-specific probe that works for all members of the genus and that does not cross-react with members of other genera. To minimize the chances of a species-specific probe failing to detect a particular strain of a species, as many strains as possible from a wide geographic and in some cases the host range should be examined. Finally, in order to confidently employ molecular diagnostics, the assays should undergo rigorous validation against established diagnostic protocols. Several examples of how these techniques are used to detect aquatic pathogens will be discussed.

Issues Associated With An OIE Reference Laboratory For Shellfish Disease Diagnosis

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The Office International des Épizooties (OIE) is a Paris, France-based organization whose aim is to assure the sanitary safety of international trade in aquatic animals including fish, molluscs and crustaceans. This is accomplished by identifying diseases of concern, called "reportable diseases," and by developing health measures implemented by member countries that prevent the transfer of reportable diseases while avoiding unjustified barriers to trade. The OIE approves a Reference Laboratory for each reportable disease, which is responsible for developing and standardizing diagnostic tests and providing diagnostic support for international colleagues. The OIE also appoints a Reference Expert for each reportable disease, who is responsible for providing advice to the OIE. The OIE publishes three documents—1) the Aquatic Animal Health Code that lists all reportable diseases and explains the steps necessary to be designated a disease free country, and reporting requirements should a disease outbreak occur; 2) the Manual of Diagnostic Tests for Aquatic Animals, which includes OIE-approved diagnostic methods developed by the Reference Laboratories; and 3) Annual Reports of OIE Reference Laboratories, which document all research and diagnostic support provided by reference laboratories. Accurate and rapid diagnosis for reportable diseases is critical to prevent spread of pathogens and also because of the economic implications associated with possible trade restrictions. DNA-based diagnostics are increasingly being used, but interpretation can be problematic. Most countries require confirmation of identification of reportable disease outbreaks by the Reference Laboratory for that disease agent. Some case examples will be discussed.

USDA/APHIS And Veterinary Diagnostics

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One role of the Animal Plant Health Inspection Service (APHIS) in support of the aquaculture industry is to facilitate the movement of aquatic animals and products in interstate and international commerce by providing health certification services. In many cases the importing country or facility requires that the surveillance or diagnostic testing procedures be conducted in a USDA, APHIS approved laboratory. Any Federal, state, university, or private laboratory is eligible for APHIS approval. The procedures for designating laboratories as qualified to conduct diagnostic testing of aquatic animals, embryos, ova and semen intended for export are outlined in Veterinary Services memorandum 567.2. In brief, the approval process requires consultation with your state's Federal Area Veterinarian in Charge, including a laboratory site visit, submission of laboratory documentation (specifics outlined in memorandum), and review of the laboratory's testing protocol(s) for scientific merit and conformity to internationally recognized standards. Internationally recognized standards can be found in the Office International Des Epizooties (OIE) Diagnostic Manual for Aquatic Animal Diseases and the OIE International Animal Health Code. In accordance with the OIE International Animal Health Code, the United States is required to report to the OIE any aquatic diseases that are on the list of OIE notifiable diseases. The official contact point for reporting to the OIE on notifiable diseases in the United States is APHIS. Any diagnostic laboratory that identifies or detects OIE notifiable diseases should report them to the Federal Area Veterinarian in Charge in their state.

Standardization And Implementation Of Molecular Diagnostics Used In The United States Fish And Wildlife Service's National Fish Health Program

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Until recently, fish disease inspection and diagnosis performed by the U.S. Fish and Wildlife Service (USFWS) relied on standard protocols published in the American Fisheries Society- Fish Health Section's "Bluebook". When the USFWS implemented the National Wild Fish Health Survey in 1997, however, the decision was made to include recent advances in molecular diagnostics as corroborative tests to accepted detection methods. This deviation from standard "Bluebook" protocol presented the nine USFWS fish health laboratories with the opportunity to field test molecular techniques developed for detection of pathogen genome segments from fish tissues. The necessity to impose strict standardization was realized and accomplished with the publication of the USFWS Wild Fish Health Survey Laboratory Procedures Manual in 2000: a detailed set of standard operating protocols for every procedure and assay employed in the Wild Survey. The impressive quality of this manual lead the USFWS Fish Health Program to evolve a complete set of protocols and procedures employed at all nine fish health centers in every aspect of operation. In addition, it has led to a successful collaboration with the AFS-FHS to revise the Bluebook with the addition of Standard Procedures for Aquatic Animal Health Inspections (2003, 5th edition). Molecular techniques have now become a Bluebook standard for confirmatory testing. This presentation will discuss the advantages and limitations of using polymerase chain reaction (PCR) technology as a corroborative test in addition to previously established standard protocols for aquatic animal health inspection and diagnostics. In addition, the good and bad implications of using PCR beyond the scope of these standards is discussed, with particular reference to the use of PCR to screen fish samples for pathogens such as Infectious Salmon Anemia virus in wild fish.

Species Specific Molecular Probe Investigations Of "White" Diseases Of Corals

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A fluorochrome-labeled molecular probe specific to *Aurantimonas coralicida*, the bacterial pathogen of the coral disease white plague type II (WPII), was constructed for use in the investigation of coral disease etiology. The probe was optimized and validated in the laboratory using pure cultures. It was designed based on a unique subset of the complete16S rRNA gene sequence obtained using a pure culture of the pathogen. The pathogen specific probe was labeled with the fluorochrome fluorescein isothiocyanate. As a control, a second, universal eubacterial probe (EUB 338) was constructed and labeled with a different fluorochrome (tetra-methyl-rhodamine isothiocyanate). The probes were used in analyses in conjunction with fluorescent *in situ* hybridization (FISH). We are currently using the probe to investigate the identification of pathogens associated with the "white" diseases of corals. "White" diseases are a suite of diseases characterized by coral tissue lysis that is associated with either a thin band of advancing, completely bleached (i.e. white) coral tissue, or a thin line that separates apparently healthy coral tissue from freshly exposed (white) coral skeleton. This suite of diseases includes three forms of white plague (types I, II, and III); two forms of white band (types I and II); white pox; patchy necrosis; shut down reaction; and bacterial bleaching. Of these, associated primary pathogens have been isolated and characterized for only three (white plague type II, white pox, and bacterial bleaching). It has been postulated that the same microorganism may cause some of these diseases, particularly those for which no pathogen has been identified. Through use of the probe on coral tissue samples collected from corals infected with each of the white diseases we are investigating the potential involvement of *A. coralicida,* either to document it's involvement or to rule it out as a potential causative agent.

Assessment of Bacterial Communities Associated with White Plague-Infected *Montastraea annularis* Corals from St Croix

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The coral disease white plague type II (WPII) threatens scleractinian corals and the reefs they support in the Caribbean and Western Atlantic. The disease is particularly virulent and kills coral tissue at a rate of 2 cm per day. It has been documented in the waters of the Bahamas, Florida, Puerto Rico and the U.S. Virgin Islands (USVI), and affects at least 17 species of coral. Due to the virulence of WPII, it can kill entire coral colonies within days and can alter the structure, composition and function of coral reef ecosystems. Although studies have concentrated on identifying the etiological agent, until recently the microbial community structure of WPII- infected tissue and healthy coral tissue had not been investigated. Furthermore, most investigations into the disease have utilized culture-based techniques, which may not provide an accurate assessment of the entire community. The goal of this research was to compare bacterial communities between healthy and diseased coral, and to determine if the communities associated with the coral disease WPII are the same among different infected *Montastraea annularis* coral colonies. Samples were collected from the water column, apparently healthy, and infected *M. annularis* corals at Sprat Hole reef in St. Croix, USVI. Samples were cultured for enumeration and to provide cellular characteristics and bacterial isolates. Additionally, whole-community DNA was extracted from samples for use in the molecular fingerprinting technique, Length Heterogeneity Polymerase Chain Reaction (LH-PCR). The LH-PCR technique provides information on the composition of the bacterial community and abundance estimates for each of the community members. The combination of culture-based approaches and molecular tools such as LH-PCR, provides a more complete assessment of the bacterial communities associated with the apparently healthy tissue and the diseased coral state.

Amplicon Length Heterogeneity - A Tool To Investigate Coral Microbial Communities

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A microbial community is an assemblage of organisms, genes and gene functions that are linked in a complex web that sustains them as a unit. It is unlikely that one microbe has all the genes necessary to survive in any environment or adequately represents the total microbial community function. Many of the basic concepts for developing a rational ecological approach to microbial ecology have been difficult due to methodological limitations. However, these limitations are rapidly decreasing with expanded use of molecular methods combined with new computational tools. There is an urgent need to understand what happens inside complex interactions that exist in the "black box" of bacterial diversity because shifts in bacterial communities can signal onset of ecological stress or episodic diseases. Development of new genomic tools allows for design of community-level studies that will interrogate the finer details of the biological components of a given habitat. One such molecular tool is amplicon length heterogeneity (ALH) DNA profiling of bacterial communities. This technique interrogates the variable domains of the ribosomal small subunit genes (SSU rDNA), and separates these amplicons on high-resolution genetic analyzers. The assays are based on natural variation in sequence lengths of specific regions of DNA. Lengths are independent of restriction enzyme recognition sites thus eliminating problems associated with other techniques. Data are phylogenetically relevant because the amplicon lengths can be associated with specific taxonomic sequences archived in databases. Black Band Disease (BBD) of corals consists of a microbial biofilm community dominated by cyanobacteria. The disease has impacted the world's coral reefs. It was suggested that the BBD community included disease-associated microbes and community members associated with corals in the non-disease state. Preliminary data from ALH profiling supported this idea and indicated that the BBD community is dominated by amplicons associated with members of the alpha-proteobacteria. Amplicons representing the members present in the non-disease state community are present but at lower abundance. With bioinformatics tools, we are conducting a 'virtual' molecular analysis of BBD. Cloning and sequencing of BBD samples from different geographical regions and coral species will verify these *in silico* inferences.

Recent Studies On Yellow Band Disease Of Scleractinian Corals

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Yellow band disease (YBD) affects massive corals in the Caribbean, particularly species in the genus *Montastrea*. Surveys in Bonaire indicated that as many as 90 % of the *Montastrea* species were affected with the disease. Measurements of the progression of tissue lesions were about 0.6 cm/month, but this can increase with temperature and nutrient loads. Within tissue affected with YBD, fewer zooxanthellae, lower chlorophyll concentrations and fewer dividing zooxanthellae cells were reported. Bacteria isolated from YBD affected tissue and inoculated onto healthy tissue produced typical signs of YBD. Different bacterial strains, all in the genus *Vibrio*, could induce YBD signs in healthy tissue, but a combination of strains were more effective. This evidence indicated that a differential zooxanthellae strain response to specific *Vibrio* strains might be responsible for YBD.

Differential Response Of Zooxanthellae To Bacterial Toxins That Produce Bacterial Bleaching In Stony Corals

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Vibrio shiloi is the species responsible for bacterial bleaching in some stony corals in the Mediterranean Sea. This bacterium produces extracellular toxins that inhibit photosynthesis of zooxanthellae and ultimately, lyse the symbiotic algae. It has been hypothesized that this bacterium might play a role in bleaching of stony corals from the Caribbean and other areas. To evaluate the possible effects of *V. shiloi* species in bleaching events from different parts of the world, zooxanthellae were extracted from corals of the Mediterranean Sea, Red Sea, and the Caribbean. These zooxanthellae were then exposed to the extracellular toxins produced by this microorganism. Our results showed that the zooxanthellae extracted from different corals differed in their responses to the extracellular materials produced by *V. shiloi*. For example, *V. shiloi*'s Toxin P more strongly affected zooxanthellae extracted from *Oculina patagonica* than from zooxanthellae isolated from Acroporid corals from the Red Sea and Milleporid corals from the Caribbean. These differences might be the result of variation in the tolerance to ammonia of different zooxanthellae clades or even species. It might also be a result of differences in the specific binding capacity of Toxin P to the algal membranes. These results might have implications in the tolerance of some corals to bacterial bleaching. Tolerance can also be the result of host-specificity of the bacteria and the coral, because the bacteria have to overcome the barrier of the coral before adhering to the zooxanthellae.

Histopathology Of The White Pox Disease Of The Caribbean Elkhorn Coral *Acropora palmate*

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White pox disease of the Caribbean elkhorn coral *Acropora palmata* is caused by the enterobacterium *Serratia marcescens.* The disease has contributed to catastrophic declines in elkhorn coral populations in the Florida Keys, with losses averaging 88% between 1996 and 2002. White pox-affected and apparently healthy coral tissues were collected from diseased colonies of *A. palmata* in August 1998 at Looe Key Reef, Florida. The tissues were processed for histopathological examination with light microscopy. Cellular and tissue degeneration was observed in both apparently healthy and diseased coral tissues. White pox disease is associated with (1) rounding of granular gland cells in the mesenterial filaments, (2) atrophy of the coenosarc, (3) necrosis of the coenosarc, oral disk, gastric cavity, and mesenterial filaments, and (4) disruptions in the gastric cavity. Most lesions were concentrated in the coenosarc tissue. There was no significant difference in the number or type of lesions present in diseased versus apparently healthy samples. While the frequencies of 41% of the abnormalities showed a significant difference between apparently healthy and diseased tissues, half of these abnormalities were more common in diseased tissues. The overall extent and severity of lesions was significantly different between apparently healthy and diseased tissues. However, only seven of the 26 individual lesion types showed a significant difference in extent and severity between apparently healthy and diseased tissues. The similarities between apparently healthy and diseased tissues suggest that colonies affected by white pox sustain a whole-colony reaction to infection.

Update On The International Registry Of Coral Pathology

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The International Registry of Coral Pathology (IRCP) was recently established by the National Oceanic and Atmospheric Administration at the Center for Coastal Environmental Health and Biomolecular Research in Oxford, MD in affiliation with the Coral Disease and Health Consortium (CDHC). The coral registry is designed to offer a safe, permanent repository where the scientific community can deposit, retrieve, and study archived materials. The IRCP has expanded its tissue archives to over 140 specimens this year through the accession of healthy and diseased coral samples representing sixteen species and eleven locations. The coral registry has also provided histology and histopathology support in collaborative coral disease investigations. For example, the coral registry participated in the development of collection and fixation protocols during a coordinated CDHC response to a summer coral mortality event in the Florida Keys. The IRCP prepared microslides from collected samples and organized an expert histopathology review panel. Similar research support is being provided in an ecotoxicology investigation of coral reefs in Bermuda. Further, IRCP has initiated several technical studies to develop and/or apply new methods to the study of corals and to help develop standard coral histology protocols. Six fixatives, four decalcification solutions, and several stains are being compared in a study to determine optimal preservation of coral tissues. Petrography methods are being evaluated in other studies to permit the examination of calcium carbonate skeletons routinely removed during decalcification. The archives and investigations of the coral registry are designed to support CDHC goals to identify coral disease etiologies, develop diagnostic criteria, and standardize terminology and descriptive pathology.

Shifting the Paradigm For Coral Reef Health Assessment

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In the last five years a new paradigm has evolved for coral health assessment, which embraces the use of science and technologies that can move us from a descriptive science (e.g. a phenomenon has occurred) into the realm of mechanistic science (e.g. how has the phenomenon occurred). Coupling the principles of biomedical science (e.g., biochemistry, cell physiology and functional genomics) with traditional measures of ecosystem health, produces integrated strategies that can (1) identify a stressor(s) affecting marine biota, (2) distinguish among different physiological conditions (3) elucidate mechanisms leading to adverse health effects resulting from interactions between organisms and their environment, and (4) predict the consequences of these interactions. This approach provides a means to understand mechanisms of action at a molecular, cellular, organismal or population levels that determine responses of organisms to their environment, and allows marine scientists to address questions such as: How do marine resources respond to a changing environment? How can one determine when a system has exceeded its plasticity in responding to a changing environment and determine its fate? How can the factors affecting coral reefs be identified? Answers to these questions will enable identification of 'cause and effect' relationships so that recognition of the 'smoking gun' of coral decline may give new insights for devising wise management options.

The Prophenoloxidase Activating System Of The Lobster *Homarus americanus*

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Previous research has implicated the prophenoloxidase (proPO) system in non-self recognition and host defense mechanisms of certain anthropods. This system has been most thoroughly described in insects and crustaceans, particularly shrimp, crabs and crayfish. However, proPO has not previously received much study in the commercially important American lobster. *In vivo* proPO and serine proteases are localized in hemocyte granules and are released from the cells during phagocytosis or other situations that trigger degranulation. In the plasma, the proPO system is activated, producing factors that enhance phagocytosis, encapsulation, coagulation, fungistatic activity and melanization. Using hemocyte lysate supernatant (HLS) and trypsin as activator, the presence of a proPO activating system was shown for *Homarus americanus*. A low-level proPO activity found in HLS was probably due to endogeneous serine protease. *In vitro*, proPO activation was strongly stimulated by trypsin in a dose- and time-dependent manner. However, microbial non-self determinants such as bacterial lipopolysaccharides (LPS) and fungal ß1,3 glucans failed to activate lobster proPO in HLS. Other potential activating procedures, such as increasing incubation temperature or preheating HLS, were not effective. Studies are in progress to determine if LPS and/or ß1,3 glucans promote hemocyte granulation rather than directly activating cell-free components of the proPO system. It seems that the proPO system of lobsters differs from that described in other crustaceans; however, such differences in mechanistic details are to be expected, based on comparative studies of proPO activation in marine crabs and freshwater crayfish.

Identification Of Tumor Necrosis Factor (TNF) And TNF-Receptor Superfamily Members Expressed In Rainbow Trout (*Oncorhynchus mykiss*)

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Tumor necrosis factor (TNF) and its receptor are each prototype members of large gene superfamilies. Since the discovery of tumor necrosis factor and lymphotoxin α, 17 additional ligands and 29 cognate receptors have been identified in mammals. Many members of the TNF and TNF-receptor superfamilies play important roles in coordinating the proliferative and protective functions of pathogen-reactive cells. Some receptors function as decoy receptors as they lack a transmembrane domain and interfere with ligand signaling. These large superfamilies may exist in rainbow trout as two ligands, one receptor, and one decoy receptor have been identified. We searched a database of rainbow trout expressed sequence tags to identify additional genes that may belong in the ligand or receptor superfamilies. We have identified and sequenced two cDNA clones that have homology to TNF ligands and nine cDNA clones that share homology with TNF receptors. Of the putative receptor genes, four cDNA clones may be decoy receptors as they lack a predicted transmembrane domain. RNA expression analysis of these genes in *Yersinia ruckeri* bath vaccinated and challenged fish is underway. These data indicated that a number of TNF ligand and receptor superfamily members were expressed in rainbow trout and suggested that some of these genes might have arisen early in vertebrate evolution.

Striped Bass (*Morone saxatilis*) *Nramp* Is Inducible *In Vitro* After Exposure To LPS And *Mycobacterium marinum*

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Mycobacteriosis in Chesapeake Bay striped bass (*Morone saxatilis*) is an ongoing disease problem with potentially important economic implications for the commercial and recreational fishery. The natural resistance-associated macrophage protein 1 gene, *Nramp1*, is responsible for innate resistance to mycobacterial infections in mice. *Nramp1* is inducible by treatment with antigenically unrelated intracellular macrophage parasites and by synergistic activity of bacterial lipopolysaccharide (LPS) and interferon-?. The striped bass homolog, *MsNramp*, is strongly induced in peritoneal exudate cells (PE) after intraperitoneal injection with *Mycobacterium* spp. *in vivo*. Unlike its mammalian homolog, *MsNramp* is expressed ubiquitously, but at variable levels depending on tissue type. The purpose of the present study was to investigate short-term *in vitro MsNramp* expression and reactive oxygen intermediate production in primary cultures of adherent anterior kidney cells (AK) and PE after exposure to LPS, or live- or heat-killed (HK) *Mycobacterium marinum*. Peritoneal exudate cells expressed significantly higher levels of *MsNramp* at 4 and 24 hours post-treatment with live and HK *M. marinum.* *MsNramp* response to LPS was dose-dependent in these cells, with maximum expression at 4 hr and 20 μg/ml LPS. Treatment of PE with LPS caused an increase in intracellular superoxide anion (O_2^-) levels, whereas treatment with live *M. marinum* caused a significant depression. Adherent AK responded to LPS with increased ROI and *MsNramp* production, but were not induced or suppressed relative to controls by mycobacteria. These results suggested that *MsNramp* might be regulated similarly to mammalian *Nramp1*. This study represents the first report of induction of an *Nramp* gene by mycobacteria *in vitro* in a poikilothermic vertebrate, and supports reports of *Nramp* induction by LPS in teleosts.

The Response Of The Immunome Of Haddock (*Melanogrammus aeglefinus*) To Vaccination

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Atlantic cod (*Gadus morhua*) are reported to have an absent immunoglobulin (Ig) response to vaccination. We have found that another gadid, haddock (*Melanogrammus aeglefinus*), also does not produce specific Ig to a variety of T_D antigens. To determine the mechanisms of this we investigated the haddock immunome (Ig variable (V_H), Diversity (D) or Joining (J_H) heavy chain gene Ig rearrangements), in vaccinated and control animals. We obtained >60 sequences from each group of naïve or vaccinated fish and these were translated at EXPASY and aligned by CLUSTAL-W. Identity matrices and entropy plots were constructed using BioEdit. Almost every sequence had a unique VDJ structure indicating that they were derived from independent B-cell rearrangements. Identity matrices showed most of the V_H shared >80% identity at the nucleotide level and therefore belonged to a single V_H family, which showed greatest homology to human V_H family II. There was no preferential usage of any V_H, D or J_H in vaccinated haddock compared to unvaccinated controls. The immunomes of unvaccinated and vaccinated haddock were more diverse than that reported for Atlantic salmon (*Salmo salar*) survivors of an *Aeromonas salmonicida* challenge. Entropy plots showed a greater diversity in all three Complementarity Determining Regions (CDRs) and in all groups CDR3 was relatively short (8 amino acids), a feature shared with surviving salmon. It appears that, *in lieu* of an antigen-specific Ig response upon vaccination haddock, and perhaps all gadids, utilise a pre-existing pool of Ig that has diverse CDR1 and CDR2 as well as a short CDR3, characteristics seen in Atlantic salmon survivors of *A. salmonicida* infection, but not in naïve controls. These data suggest that this Ig pool may be somewhat specific and not "sticky", as has been suggested in Atlantic cod. Furthermore as haddock have a relatively diverse immunome, they must also have a relatively diverse genome, yet a limited proteome has been described, and functional studies have shown limited, if not absent, Ig function. Our data suggest that the paucity of specific Ig production upon vaccination is due to an inability to access the B-cell repertoire, rather than a poor B-cell repertoire itself.

Potency And Shelf-Life Of A *Streptococcus agalactiae* Vaccine In Nile Tilapia (*Oreochromis niloticus*)

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Group B streptococcal infections have been associated with significant morbidity and mortality in freshwater, estuarine, and marine fish species. Currently, no commercial fish vaccines are available that prevent disease caused by the emerging group B streptococcal fish pathogen, *Streptococcus agalactiae*. Thus an effective vaccine for *S. agalactiae* could help decrease associated mortalities due to *S. agalactiae* infection. Juvenile Nile tilapia (*Oreochromis niloticus*) were used to test the efficacy of a killed vaccine composed of formalin-killed *S. agalactiae* cells and concentrated extracellular products. Previous studies showed that fresh vaccine provided significant protection against *S. agalactiae* challenge. Our objective was to determine whether vaccine stored at 4ºC for one year remained efficacious. Tilapia were inoculated with one year-old vaccine by intraperitoneal (IP) injection, and control tilapia were inoculated IP with tryptic soy broth (TSB). All fish were then held at approximately 32ºC. At 31 days post-inoculation, fish were challenged IP with $1.7x10^4$ CFU *S. agalactiae*/fish. Fish were monitored for clinical signs and mortality for 14 days. Blood was also sampled from control and vaccinated fish on days 0 and 31 for antibody production. In our previous studies, inoculation with fresh vaccine significantly helped prevent development of clinical signs and reduced post-challenge mortalities, yielding a relative percent survival (RPS) of 80. The vaccine held for one year only produced an RPS of 29 and allowed development of clinical signs. The year-old vaccine induced significantly increased antibody production in vaccinates as compared to controls. However, this production was significantly lower than that produced with fresh vaccine. This decreased antibody production may account for the decreased RPS. This study suggested that shelf life of the vaccine <1 year when stored at 4ºC, indicating that a fresh preparation of the vaccine should be utilized to obtain the best efficacy. Because it is desirable to have vaccine available for multiple tilapia production cycles, other vaccine preparations (lyophilized) or storage considerations (freezing) should be assessed.

Immune Responses Of Channel Catfish Against *Ichthyophthirius multifiliis* After Immunization With Live Theronts And Sonicated Trophonts

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The ciliated protozoan *Ichthyophthirius multifiliis* Fouquet (Ich) causes high mortality in fish and leads to heavy economic loss in aquaculture. The Ich infection is difficult to control with chemical treatments because the parasite penetrates into fish skin and gill. Fish that survived an Ich infection acquire immunity against reinfection by the parasite. Vaccination against the parasite can be used as an alternative to chemical treatments. This study determined the humoral immune responses and host protection of channel catfish *Ictalurus punctatus* (Rafinesque) against *Ichthyophthirius multifiliis* (Ich) after immunization with live theronts and sonicated trophonts, with an emphasis on assessing the cutaneous immune response. Immunizations with live theronts or sonicated trophonts were done by both bath immersion and intraperitoneal (IP) injection. Cutaneous and serum immunoglobulin (Ig) levels and anti-Ich antibodies were measured 12 and 21 days post immunization with the enzyme-linked immunosorbent assay and theront immobilization assay. The level of Ich infection and survival of catfish were determined after theront challenge at a dose of 15,000 theronts per fish for 1 hour. Cutaneous and serum anti-Ich antibodies were significantly higher in fish immunized with live theronts by immersion or IP injection or with sonicated trophonts administered by IP injection as compared to fish immunized with sonicated trophonts by immersion, with bovine serum albumin by IP injection, or non-immunized controls. Host protection was noted only in fish immunized with live theronts by immersion or IP injection or with sonicated trophonts by IP injection. There was a positive correlation between higher levels of anti-Ich antibodies and host survival in the immunized fish. Specific anti-Ich antibody levels appear to be a better indicator of Ich tolerance than total non-specific Ig.

Bothriocephalus acheilognathi: The Australian Experience

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The Asian fish tapeworm, *Bothriocephalus acheilognathi*, was originally a parasite of carp and related cyprinids in the Amur River basin of China. It has now been distributed around the world due to the translocation of its hosts for aquaculture, ornamental display, and weed control. In the process, the list of fish species that it infects has grown to over 50, representing six orders of fish and one amphibian. It is this extraordinarily low host-specificity at all life history stages that has allowed the worm to establish in non-native habitats, despite the complexity of its life cycle. Australia has a depauperate freshwater fish fauna of just 180 species with a high degree of familial and generic endemism. I collected over 1400 fish from a range of sites in coastal and inland drainages of eastern Australia. *Bothriocephalus acheilognathi* was recorded from all well-sampled native hosts that were collected sympatrically with carp. The worm was not recorded from any sites where carp were absent. These patterns, combined with the fact that *B. acheilognathi* rarely matures in native fishes, suggested that a reservoir of infection in carp was required for infections to persist in native fishes. Given the potential pathogenesis to hosts (stenosis, eosinophilia) it appears that parasite-mediated competition may occur between exotic and native fish species. *Bothriocephalus acheilognathi* appears to be a silent and underestimated mechanism by which carp can damage non-native ecosystems.

Bolbophorus Infections In Catfish: Identification, Impact and Treatment

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Bolbophorus is a genus of trmatodes that infects pelicans, rams-horn snails and fish. The trematodes have a hind- and forebody, lateral pseudosuckers around the oral sucker, a holdfast organ, interconnecting excretory channels, and a clear parasite cyst within the host cyst. The taxonomy of these parasites is in transition and there are at least four species. Prior to 2002, most of these trematodes were identified as *B. confusus* , but that nomenclature is now restricted to one species from Europe. In the United States, *B. damnificus* and other distinct species (*B.* spp.) are found in fish. *Bolbophorus damnificus* occurs in the flesh of channel catfish and fathead minnow, while other *B.* spp. occur in cyprinids, centrarchids, and percichthyids. *Bolbophorus damnificus* causes serious losses in the catfish industry. The trematode first showed up on a catfish farm in Louisiana in 1994 and has since been found on >50 catfish farms in Arkansas, California, Louisiana, and Mississippi. The trematode continues to spread and losses continue to occur but, in the last two years, the number of case reports dropped. This was partially attributed to the ability of farmers to privately diagnose and implement management and control measures. Pond shoreline treatments of copper sulfate and hydrated lime as well as the stocking of black carp have lessened the impact of the parasite. With the probable listing of black carp as an injurious species, greater impetus is placed on chemical treatment. Copper sulfate treatment effectively reduces rams-horn snails that host *B. damnificus.* Ten pounds (4.5 kg) of copper sulfate applied along 250 ft (76.3 m) of shoreline in a 6 ft (ca 2 m) wide band reduced snail survival to about 5%. The treatment lost effectiveness below 21°C, but was very effective even at ½ the copper sulfate rate at temperatures of 31°C and higher. The hydrated lime treatment, although reported as effective, is not well defined or tested: recommended treatment rate per shoreline length and treatment width varied, influence of temperature on the treatment was unknown, and treatment results were not published. Tests were initiated to define the effectiveness of the hydrated lime treatment. Initial results indicated that treatment was effective if 90 lbs (27.5 kg) of hydrated lime was applied along 100 ft (30.5 m) of shoreline in a 3 ft (ca 1 m) wide band. The effectiveness of hydrated lime, when applied at 31°C, was dramatically higher than when the same treatment was applied at 25±2°C. Other tests are planned.

Preliminary Study On The Mitochondrial Genome Of The Tapeworm *Bothriocephalus acheilognathi*

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The tapeworm, *Bothriocephalus acheilognathi* has been a problem with aquacultured grass carp and golden shiners for about 30 years and has recently been reported in many other cyprinids**.** Several states restrict the importation of fish with *B. acheilognathi*, therefore, identification and detection of these worms are very important. Because definitive identification methods vital to the inspection process do not exist, a study was initiated to develop such methods. Molecular genetic approaches using both nuclear and mitochondrial genes have been used in studies on the phylogenetic relationship of closely related organisms including several species of cestodes. Past studies on the internal transcribed spacer of ribosomal DNA (ITS rDNA) have determined genetic variation in *B. acheilognathi* in different fish hosts. It was found that there were three closely related genotypes with certain degrees of host-specificity. Metazoan mitochondrial sequences are known to evolve rapidly, but gene arrangements will remain unchanged for long evolutionary periods of time. Therefore, information on the mitochondrial genome is important in understanding the evolutionary relationships of cestodes. In this study we will attempt to look at the mitochondrial sequences of *B. acheilognathi* using the cytochrome B region and several primers designed for long chain PCR. Samples of *B. acheilognathi* from were collected from grass carp at a private aquaculture facility in Arkansas.

Cestodes Of Elasmobranches: Friends Or Foes

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Cestodes of sharks and rays exhibit amazing diversity of morphological forms represented by four orders and approximately 900 species. Three orders, the Diphyllidea, Lecanicephalidea and Trypanorhyncha, are known only from elasmobranches; the fourth and most diverse order, the Tetraphyllidea, primarily parasitize sharks and rays, but a few species parasitize holocephalans. Essentially all adult cestodes in these orders parasitize their host's spiral intestines. Neither the evolutionary relationships among the tapeworms of elasmobranches, nor the relationships between the tapeworms of elasmobranchs and the 10 orders of tapeworms that parasitize other vertebrates are well understood. It seems clear that the four major tapeworm lineages that parasitize elasmobranches are not each other's closest relatives. Rather, it appears that several unrelated groups of tapeworms have become associated with sharks and rays. Second, it is likely that the associations between the majority of these groups of tapeworms and their elasmobranch hosts are remarkably ancient, which possibly accounts for their incredible host specificity. Investigation of the mode of attachment of a diversity of tetraphyllidean tapeworms reveals that the interface between each tapeworm and the spiral intestine mucosal surface is extremely intimate. The morphology of the tapeworm's primary attachment structure, or scolex, corresponds closely to the surface of the mucosa at the site of attachment. However, these tapeworms exhibit a variety of strategies for attachment. Whereas some species insert themselves into existing spaces offered by the normal configuration of the mucosa, other species grasp the various surface elements of the mucosa. Yet others induce changes in the mucosa that facilitate their attachment. Although it remains to be confirmed, field observations suggest that larger tapeworm species are more destructive than smaller species. This observation is interesting given that our field work has shown that some stingray species routinely host thousands of small tapeworms, but large tapeworms are uncommon parasites of these animals. Unfortunately, several major aspects of the biology of these parasites, which might provide insight into these observations, remain poorly understood. For example, a complete life cycle has yet to be determined for any of these tapeworm species.

Histopathological Examination Of Wild American Eels, *Anguilla rostrata,* Infected With *Anguillicola crassus*

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Anguillicola crassus is a parasite that is native to the Japanese eel (*Anguilla japonica*) and has been introduced in the European eel (*Anguilla anguilla*) and American eel (*Anguilla rostrata*). Typically the introduction of a parasite will have a greater effect on the naïve rather than its native host. This has been the case in wild and cultured European eels and cultured American eels. Pathogenesis of *A. crassus* in eels has included enlarged abdomens, skin ulcers on the posterior part of the abdomen, red and swollen anus, thickening of the entire swim bladder wall through the proliferation of epithelial cells and fibrosis, lymphocytic infiltration of the swim bladder tissue, dilation of blood vessels in the swim bladder wall, hemorrhaging in the swim bladder, and swim bladder rupture. We describe the histopathology of *A. crassus* in wild American eels from the Carlls River in Babylon, NY and compare it with these previous studies. This is the first examination of the pathogenesis of *A. crassus* in wild American eels.

The State Of Science Of Leech Parasites Of Fish

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All fish leeches are in the family *Piscicolidae*. Very few phylogenetic analyses have been conducted on the fish leeches, but recent molecular phylogenetic analyses, based mainly on European taxa, suggested that the family was monophyletic. A larger analysis that will include taxa from around the world is underway at VIMS. Fish leeches occur in both freshwater and marine environments, but they are far more diverse in marine environments where they occur worldwide. Approximately 95% of the 65 recognized genera occur in the ocean. Leeches are not considered to be important pathogens of fish themselves, although in unusual circumstances they can cause problems. However, fish leeches are well-documented vectors for pathogenic haematozoa. Fish leeches are difficult to identify and this has hindered research on the biology of many species, including their vector role. Taxonomic research on fish leeches, funded by the National Science Foundation PEET program (Partnerships to Enhance Expertise in Taxonomy), is underway at VIMS. The intent of this program is to produce web-based keys to the world fauna and to train students in leech taxonomy. Such keys should help other researchers identify leeches and this may result in increased attention to the group. The freshwater leech *Myzobdella lugubris* often causes lesions in the mouth of a variety of freshwater fish, including recent problems in largemouth bass in Michigan.

Molecular Characterization Of The Chlamydia-Like Bacterium Associated With Epitheliocystis In Arctic Char (*Salvelinus alpinus*)

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Epitheliocystis is used to describe gill disease associated with infection by gram-negative, intracellular, chlamydia-like or rickettsia-like bacteria. Histologically, the agent forms intracytoplasmic, basophilic, granular inclusions in gill epithelial cells. For Arctic char to become a viable commodity, disease identification and control needs to continue to be a major focus of the industry. As the bacteria associated with epitheliocystis are yet uncultured, molecular characterization was begun by amplification of 16S rRNA genetic sequences directly from infected gill. Samples were taken for histopathologic, ultrastructural analysis and nucleic acid studies from Arctic char during outbreaks of epitheliocystis. Histopathology of epitheliocystis was characterized by intracytoplasmic inclusions in gill epithelial cells with varying degrees of proliferative branchitis. Special stains, e.g. Gimenez, Macchiavello and Lendrum's techniques, all reacted positively with inclusions, which was not the case in Atlantic salmon. Transmission electron microscopic analysis of infected gill epithelial cells revealed intracytoplasmic inclusions containing round to elongate reticulate bodies embedded in a granular to fibrillar electron-dense matrix without distinct dense-core cells. Immunogold labeling using an antibody to chlamydial lipopolysaccharide demonstrated moderate labeling of membranes and cytoplasm of reticulate bodies. Order-specific chlamydiales primers were used in PCR-based experiments to amplify a 300-bp 16S rRNA gene sequence considered to be a "signature" region. The PCR products were sequenced directly and assembled to yield an overall consensus for future phylogenetic studies. This characterization is the first step to better understand the biology of this agent.

Prevalence Of Walleye Discrete Epidermal Hyperplasia And Walleye Dermal Sarcoma By Age Class In Walleyes (*Sander vitreus*) From Oneida Lake, New York

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Walleye discrete epidermal hyperplasia has been described as a seasonally limited skin disease caused by two closely related retroviruses, walleye epidermal hyperplasia virus type 1 and type 2. Like walleye dermal sarcoma, which is also caused by a retrovirus, the in-lake transmission patterns of this disease are unknown. We followed the prevalence of visible proliferative lesions on walleye aged 3 to 8+ years from 1997 to 2003 in Oneida Lake, New York. The percent of fish developing walleye epidermal hyperplasia lesions increased slowly with age. The observed prevalence of discrete epidermal hyperplasia does not support the hypothesis that walleye with visible hyperplastic lesions in the spring will not develop the disease again the following year. It is unlikely that all walleye of a given year class will contract discrete epidermal hyperplasia in their lifetimes as less than 20% of the fish age 8 and older were infected. This is in contrast to changes in prevalence of walleye dermal sarcoma with age, a disease that we believe eventually affects almost all exposed walleye and where prevalence decreases with age after age 6 suggesting that walleye develop immunity to the disease.

Maine Strain II: A Case History Of The Multidisciplinary Analysis Of An Emerging Infectious Salmon Anemia Virus Strain Variant

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Infectious Salmon Anemia (ISA) is an important disease of wild and farmed Atlantic salmon. Strains from Norway, Scotland and North America have been characterized by genomic sequencing of segments 2, 8 or 6. The pathogenicity of different ISAV strains was studied by infectivity trials, vaccine trials, field observations and epizootiology. Recent publications forwarded the hypothesis that varying levels of pathogenicity exist among strains, and a model has been presented to explain how host-adapted, non-pathogenic 'wild' strains may repeatedly mutate into pathogenic forms. The 2001 ISA epizootic in Maine, like simultaneous epizootics in contiguous New Brunswick, Canada, was attributed exclusively to infection with the New Brunswick strain (Maine Strain I). Recent routine surveillance detected a 'new' strain of ISAV at a farmed Atlantic salmon site about 100 km from Cobscook Bay, Maine; - site of the previous epizootic. When this abstract was prepared, this strain (Maine Strain II) was not associated with elevated mortality or typical clinical disease. Maine Strain II shared genomic and epizootiological characteristics with a strain from Nova Scotian farmed salmon (1997), which was less pathogenic and more closely aligned with European strains. The Maine Strain II, while similar to the Nova Scotian strain in some respects, had a novel genomic segment 6 sequence, which shared homology with both European and North American strains. Thus far, Maine Strain II has resisted cell culture attempts. This presentation describes the events that were used to study the Maine Strain II virus. A brief history of the known strains of ISAV is given and techniques used to determine the identity and physical characteristics of the new strain are described. Important epi factors such as the determination of environmental presence of virus, monitoring the potential spread of the virus, and putative transmission vectors such as sea lice are presented. A discussion of biosecurity as it relates both to site-level and zone-level management is included. A planned infectivity trial comparing the New Brunswick and Maine Strain II of ISAV is also summarized.

Environmental Persistence Of Infectious Salmon Anemia Virus

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The transmission of Infectious Salmon Anemia virus (ISAV) among populations at aquaculture sites is not fully understood. Carrier fish species such as brown trout (*Salmo trutta*) and sea lice (*Caligus elongatus, Lepeophtheirus salmonis*) are implicated as vectors. The major route of entry is believed to be the gills and seawater is suggested as a source for its dissemination. It is presently unclear where and for how long ISAV can survive once it is shed outside of its host into the natural environment. This study examined the ability to detect the presence of ISAV in seawater, freshwater, suspended and bottom sediments, sea lice and blue mussels (*Mytilus edulis*). We also assessed the ability of ISAV to survive within some of these sample sources. Cell culture assays and reverse transcription polymerase chain reaction (RT-PCR) were used to test samples collected from ISAV-affected sites in Canada and Maine. Detection of ISAV in recently collected seawater samples was possible by RT-PCR following virus concentration using an inexpensive seawater filtration system with a sensitivity of 30 virus particles per liter of seawater. The virus was also detected on pontoon and boat surfaces, and in sea lice and mussels by RT-PCR but not by cell culture assays. Sequencing of genomic segments from various isolates indicated that different viral strains were present at the sites that were tested. Sea lice, which tested positive for ISAV but were collected from Atlantic salmon (*Salmo salar*) that had tested ISAV negative, also indicated that there was some possibility of recent host switching. Seawater and freshwater samples were inoculated in the laboratory with ISAV at 10^3-10^4 $TCID_{50}$/mL final concentration in order to follow virus loss over time under varying conditions of temperature and microbial activity through the use of $TCID_{50}$ assays and RT-PCR. In sterile seawater, ISAV cytopathic effect (CPE) was observed for up to 3 weeks incubation at 16^{o}C and 4^{o}C; detection by RT-PCR was possible for up to 9 weeks after inoculation at both temperatures. In non-sterile seawater, ISAV CPE was not observed after 24 hours and 2 weeks incubation at 16^{o}C and 4^{o}C, respectively; ISAV was detected by RT-PCR after incubation for up to two weeks at 16^{o}C and up to 5 weeks at 4^{o}C. Determinations of ISAV longevity in freshwater, in suspended and bottom sediments, and in blue mussels are ongoing.

Critical Factors For The Establishment Of Biosafety Level-3 Aquaria

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During the past five years, two viruses never reported previously in the United States, Infectious Salmon Anemia virus and Spring Viremia of Carp virus, have been isolated from both cultured and wild populations. These incidents emphasize the need for active disease surveillance, rapid detection methods to detect their presence, and vaccines to prevent their introduction spread, and minimize their impact on aquatic populations. The study of foreign pathogens and other pathogens identified in the List A of the World Organization for Animal Health (OIE) requires biosecure laboratories and aquaria. However, currently there is only one USDA certified biosafety level 3 (BSL-3) aquarium located on the West Coast in Washington State. This limitation seriously hampers research on pathogen diagnostics, transmission and vaccine development. In addition, lack of guidelines to establish BSL-3 aquaria further complicates future efforts to develop similar facilities. In this presentation we will present the results of a review of practices followed worldwide by BSL-3 facilities. Information was collected using a structured survey and unstructured data gathering activities. Critical factors including disinfection practices required by BSL-3 aquaria to ensure strict biosecurity and containment will also be discussed.

The Acute Effects Of A Handling Stressor On Select *Oreochromis aureus* Plasma Components

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Numerous plasma components in teleost fishes are useful in demonstrating stressors and include potentially immuno-enhancing or suppressing activity. In this preliminary investigation outbred blue tilapia (*Oreochromis aureus*) were anesthetized with 2-phenoxy-ethanol and bled. Then the experimental group were revived and stressed by being held in the air and placed in a shallow bucket of water for approximately 10 minutes, and bled again. The control fish were kept under anesthesia until bled again 10 minutes after the initial bleed. Plasma samples were evaluated for lysozyme, iron, cholesterol, glucose, and ion levels. Significant differences were found between the control and experimental groups in some plasma ions and glucose. Plasma iron levels increased in all fish between the initial and final bleed with a greater increase in the control fish than in the experimental fish. Plasma cholesterol levels were significantly decreased in both groups between the initial and final bleed. No differences were found in plasma lysozyme levels. The data will be presented and possible implications related to innate immunity discussed.

Absence Of Intestinal Histologic Changes In Fingerling Channel Catfish (*Ictalurus punctatus*) Fed Raw Soybean Meal

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Considerable effort has been made to utilize alternative protein sources to replace fishmeal. Soybean meal (SBM) is a commonly utilized plant protein in fish feeds, because it is readily available and inexpensive, and has a high nutritional value. However, the inclusion of high levels of SBM in fish diets has usually produced unfavorable effects, such as reduced growth rates, reduced feed utilization efficiency, and adverse intestinal histologic changes. Many of these negative effects were attributed to the heat-labile anti-nutritional factors of SBM, and thus SBM is commonly heat-treated before it is used. The histopathologic effects of feeding heat-treated or non-heat-treated raw SBM to fingerling channel catfish (*Ictalurus punctatus*) had not been previously assessed, but were studied here. Fish were fed one of six diets: a diet containing 45% commercial soybean meal (CSBM), or diets with the CSBM replaced by non-heat-treated raw soybean meal (RSBM0), or RSBM heated in an autoclave at 130 °C and 22 PSI for 5 (RSBM5), 10 (RSBM10), 20 (RSBM20), and 40 (RSBM40) min. After 10 weeks, tissue samples were taken from the stomach, proximal intestine, distal intestine, liver, pancreas, and spleen from fish in each group for histologic examination. Mild necrotic lesions were found in the gastric glands, pancreas, and liver of fish in all the groups, including the control group. Hepatic glycogen deposition was also observed in all the groups, as was moderate hyperplasia of the bile ducts. Spleen samples had considerable brown-black pigment deposition around the splenic corpuscles and diffuse mild-to-moderate congestion in all of the groups. Generally, these histological effects appeared to be equivocal between all of the groups, and abnormalities were not noted in the proximal or distal intestines. These findings suggested that unheated 45% RSBM could be used in channel catfish feed without causing severe histological changes routinely associated with SBM utilization in fish.

Histopathological And Physiological Evidence Of Thyroid Disruption In Common Carp (*Cyprinis carpio*) From The Middle And Lower Rio Grande River Basin

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In mammals the thyroid is a discrete endocrine gland comprised of thyroid follicles surrounded by a capsule of connective tissue. The teleost thyroid lacks a capsule, and follicles are often diffusely distributed along the dorsal aorta and ventrally along the branchial arches. Ectopic thyroid follicles are occasionally observed in some fish species; however, they are often present in low numbers and follicles are morphologically similar to those in the expected regions. Here we present the presence of hypertrophic, ectopic follicles located in the posterior kidney of common carp (*Cyprinus carpio*) collected from middle and lower Rio Grande River Common carp were collected from multiple sites in the upper, middle, and lower Rio Grande between mid-October and mid-November of 1997. Occasional solitary or small clusters of follicles were found in posterior kidney samples from carp in the upper Rio Grande. They were not observed in all kidney samples, however, and were generally of expected size for thyroid follicles. Conversely, follicles observed in carp from the middle Rio Grande were numerous and generally hypertrophic. They often occurred as foci containing eight or more follicles. Follicle size was highly variable, and the columnar follicle epithelium of some follicles was hypertrophied and vacuolated. Similar observations were made in carp from the lower Rio Grande, but of lesser severity. Average size and number of thyroid follicles were determined from individual carp via image analysis (Image Pro 3.0) and this data will be presented. Total plasma thyroid hormones (T_3 and T_4) were measured using a radioimmune assay validated for common carp. The T_3 /T_4 ratios (an indicator of deiodinase activity) were less than one at most sample sites. A T_3 /T_4 ratio of 5.12 (n = 20, SE = 1.65) was determined from an aberrant site in the middle Rio Grande, which indicated that there was some physiological thyroid disruption. The putative role of contaminants is discussed.

Copper Sulfate Target Animal Safety In Channel Catfish

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A histological study was performed to evaluate the effect of waterborne exposures of channel catfish (*Ictalurus punctatus*) to copper sulfate ($CuSO_4$) as a therapeutant. Channel catfish were exposed daily for 11 consecutive days to three concentrations of $CuSO_4$ representing one, three and five times the recommended therapeutic level (2.91, 8.71 and 14.55 mg $CuSO_4$/L) based on a total alkalinity of 291 mg/L ($CaCO_3$). Copper sulfate was applied in flow through tanks and more than half the copper was reduced in 1 hour similar to the elimination rates in ponds (this is due to the rapid uptake of copper by aquatic plants or algae and the binding to organic and inorganic material). The exposed fish did not exhibit any mortality, clinical signs or gross or histological lesions in the following tissues: gill, liver, anterior and posterior kidney, spleen, axial muscle with attached skin, pyloric intestine, heart, swim bladder and cornea. The absence of histological lesions was attributed in part to the brief daily exposure to Cu^{++} (the toxic form of copper), the use of alkalinity (> 50 mg/L) to calculate of the therapeutic dose, and the moderate hardness of the well water used which is essential to maintain a normal gill function. The results of this study suggested that the use of $CuSO_4$ as a therapeutant is safe provided that the dose of copper applied is considered in relation to the total alkalinity of the water. This target animal safety study followed an approved protocol by the Food and Drug Administration (FDA) to support the data package required for the future FDA-approval of copper sulfate as an aquaculture therapeutant in the United States.